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THE PELVIS FROM FISH TO MAN: A STUDY IN PALEOMORPHOLOGY[1]

PROFESSOR WILLIAM K. GREGORY
COLUMBIA UNIVERSITY AND THE AMERICAN MUSEUM OF NATURAL HISTORY

THE BEGINNINGS OF THE PELVIS

THE following outline of the evolution of the pelvis is a by-product of the labors of many paleontologists on the phylogeny and classification of the vertebrates, and of anatomists and anthropologists on the morphology of the pelvis. For convenience and brevity the story is told in the order of evolution and not in the confused order of the historical development of the problem.

That the paired fins, including the pectoral and pelvic girdles of the earliest vertebrates, were originally of the same general nature as the median fins may now be regarded as beyond reasonable doubt. In addition to all the evidence in favor of the "finfold origin" of paired fins cited by Thacher, Balfour, Dean, Goodrich and others, we now have the fact attested by Kiaer (1924) and Stensiö (1932) that in the ostracoderms, which are the oldest known chordates of Upper Silurian and Devonian age, the pectoral lobes or projections were in series with a pair of longitudinal folds or series of dermal spines that ran backward along the ventral side of the body, converging toward the cloaca.

[1] Read before Section H, American Association for the Advancement of Science, and American Folk-Lore Society, at the Pittsburgh meeting, December 29, 1934.

Also, in the very primitive acanthodian sharks (Fig. 1) these paired fin-folds again bear spines which are

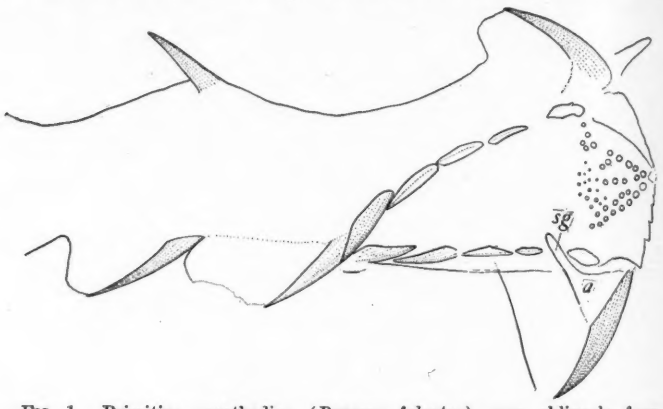

FIG. 1. Primitive acanthodian (*Parexus falcatus*), seen obliquely from below, showing paired fin-folds converging toward anal fin. After Dean, 1907.

evidently polyisomeres, or serially homologous parts; but already the process of differentiation or anisomerism is tending toward the elimination of the intermediate pairs, finally leaving only the pectoral and pelvic spines and fins as the anisomerous relicts of a once continuous series.

This is essentially the condition also in modern sharks and in their Paleozoic forerunners, but in this group the paired fins were supported by internal polyisomerous rods, which extended deep into the body and tended to coalesce proximally, giving rise to the endoskeletal pectoral and pelvic girdles. These early fins and girdles functioned chiefly as cut-waters and bilge-keels to enhance lateral stability.

The pelvic girdle of male sharks was extended posteriorly into claspers, or mixopterygia, and here we note that the pelvic girdle in nearly all vertebrates is associated with the cloacal exit and has more or less to do with reproduction.

In the most primitive fossil ganoid fishes the fins and girdles were basically similar to those of the existing

sturgeons and spoonbills, in which the pelvis has retained an apparently initial stage in the coalescence of the polyisomerous basal rods into a pelvic plate. The pelvic fins followed the pectorals in becoming paddle-like and the pelvis also became specialized as a support for the more complex musculature of the paddles. It also had several functional connections: anteriorly with the abdominal muscles, medially with the cloacal muscles and latero-ventrally with the protractor, retractor and adductor muscles of the pelvic paddles.

In primitive fishes generally the pelvic girdle contrasts widely with the pectoral girdle, both in lacking an exoskeletal layer and in not being fastened above to the axial skeleton (the pectoral girdle is fastened to the lateral corners of the skull). The pelvic girdle separates the abdominal from the caudal musculature and originally it lay wholly on the ventral surface. In the Devonian crossopterygians, which in skull structure were approaching the earliest amphibian types, the pelvis (Fig. 2) consisted of a pair of ventral rods on either side of

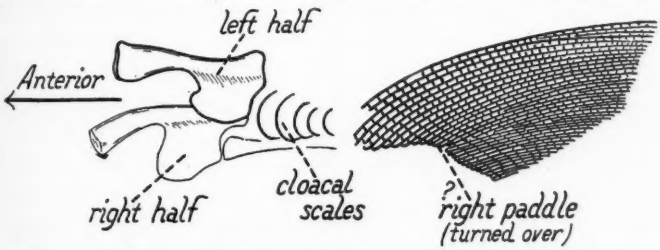

FIG. 2. Pelvic region of a typical crossopterygian (*Eusthenopteron foordi*), seen obliquely from below. After Bryant.

the cloaca, apparently representing the pubi-ischiadic rami. The rod-like anterior rami of the pelvis may have been buried in the abdominal muscles and have barely touched each other on the mid-line with only a ligamentous junction. The expanded areas may have served for the muscles of the cloaca and paddles. There is no suggestion of an ascending ramus or ilium. In the living *Neoceratodus,* a survivor of the Paleozoic lung-fishes, the

opposite pelvic rods are fused at the symphysis and there is a long median pubic process. The acetabula are pedunculate and directed backward.

That there was a time in which the dorsal blade, or ilium, began to grow up toward the ribs there can be no reasonable doubt, from what follows, but until the pelvis of the newly discovered Upper Devonian amphibians is made known, there will continue to be a hiatus in the history of the pelvis at this point.

THE PELVIS OF THE EARLIER TETRAPODS

When the piscine forbears of the Amphibia first began to use their stoutly built paddles as accessory fulcra in wriggling about on muddy flats the bony elements of the paddles, as well as the supporting girdles, were subjected to entirely new tests, which were met on the part of the organism by an anisomerous rearrangement of the pattern of the paired fins, probably in the manner dealt with in another paper. Meanwhile there was doubtless a marked increase in the size of the adductor muscles of the limbs and a corresponding expansion of the ventral portion of the pubi-ischiadic plate. As the acetabula began to turn outward rather than backward, the iliac processes grew upward, thus anchoring the future ilium in the dorsal divisions of the caudal and thoracic axial muscles. Then by further anisomerous coalescence and rearrangement, each half of the pelvis became tripartite, the parts meeting in the acetabulum in a triradiate suture. Meanwhile a somewhat analogous rearrangement in the scapular girdle resulted in a tripartite scapulo-coracoid blade. But whereas the opposite scapulo-coracoid plates were tied together by the dermal shoulder-girdle (Fig. 3), the opposite halves of the pelvis never possessed any overlying dermal plates.

The paired ilia, or dorsal processes, had already begun to grow up toward the ribs of the future sacral region; eventually they passed laterad to them, while the sacral ribs in turn began to enlarge and to be appressed toward the medial surfaces of the ilia.

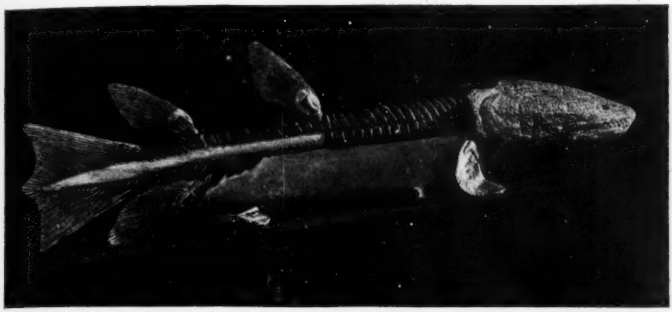

FIG. 3. Model of crossopterygian (*Eusthenopteron foordi*), based on specimens figured by Bryant.

Romer (1931) has shown that in the earliest tetrapods (Fig. 4) the lateral surface of the dorsal blade of the ilia was occupied anteriorly by the ilio-costalis and posteriorly by the ilio-caudalis muscles, only a small area, immediately above the acetabula, being occupied by the homologues of the glutei. At this time the future ilio-psoas muscle masses were chiefly ventral in position and had but little dorsal extension on to the blade of the ilium. As time went on (Fig. 5), the gluteal areas above the acetabula expanded greatly while the ilio-caudalis and ilio-costalis retreated dorsad. Finally the gluteal crest extended outward and upward to such an extent that the ilio-caudal and ilio-costal masses were found medial to the gluteal blades. Meanwhile the ilio-psoas had extended up from below, in front of the gluteal mass.

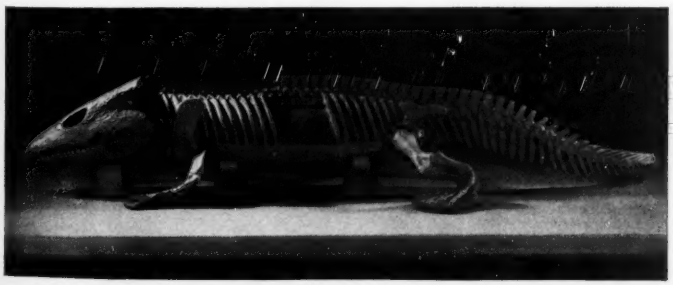

FIG. 4. Model of primitive amphibian (*Diplovertebron*) from Lower Carboniferous of Europe. Based on data published by Watson, and Fritsch.

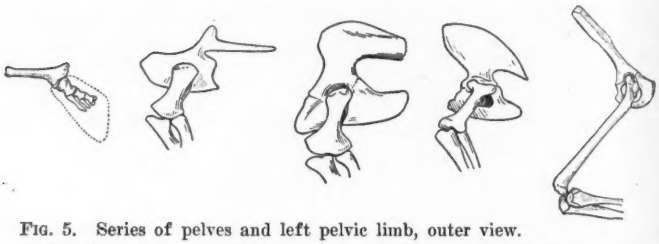

FIG. 5. Series of pelves and left pelvic limb, outer view.
A, *Eusthenopteron;* B, *Diplovertebron;* C. *Limnoscelis;* D, *Cynognathus;* E, *Opossum.*

In the earliest tetrapods the opposite pubi-ischiadic plates were considerably extended cranio-caudally, implying a wide-spreading or fan-like pubi-ischio-femoralis externus and associated muscles. To the pubis was attached the abdominal muscles and the abdominal basket, and to the pectineal tubercle of the pubis was attached the powerful ambiens muscle of the thigh, while the ischia served as a base for the ventral caudal muscles as well as for the posterior part of the cloacal muscles. Since the attachment to the sacral ribs was somewhat loose, the pelvis was braced below by a thickened symphysial junction of the opposite pubi-ischiadic plates.

THE PELVIS OF THE MAMMAL-LIKE REPTILES

Up to this point the pelvis of the typical Permian tetrapods consisted on each side of three bones, the ilium, the pubis and the ischium, arranged around a large triangular acetabulum in an approximately symmetrical or isosceles triangle (Fig. 6A). Such an arrangement was associated with a widely-sprawling posture of the pelvic limbs, in which the knees were directed more outward than forward and the feet could be brought near to the mid-line only by lateral undulations of the vertebral column. But in the early mammal-like reptiles the "isosceles triangle" of the pelvis gradually became changed into a scalene triangle by the backward displacement of the base, or pubi-ischiadic plate, and the forward inclination of the tip of the ilium. Romer (1922) has

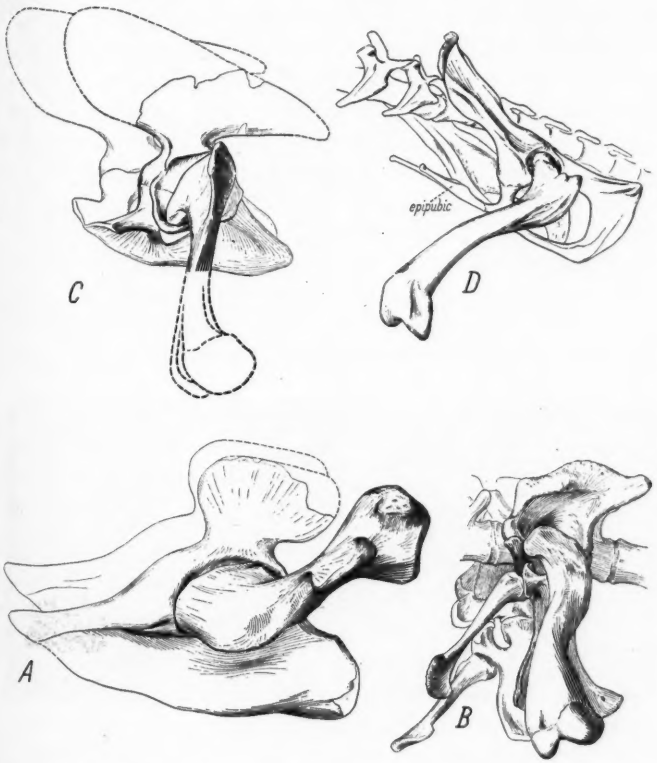

FIG. 6. Pelves and femora (left side). After Gregory and Camp.
A. Dimetrodon, primitive reptile with symmetrical pelvis adapted to crawling gait.
B. Alligator, highly specialized reptilian derivative of *A.*
C. Cynognathus, mammal-like reptile with ilium extended forward and short pubi-ischiadic plate.
D. Myrmecobius, fairly primitive mammal with ilium narrow and greatly extended forward.

shown that this process was part of a shift of the gluteal and iliac muscle masses to more advantageous positions, as the limbs reached more directly forward and the feet were brought nearer the mid-line in running. The deinocephalian *Moschops,* although somewhat far off the direct line of ascent to the mammals, shows an early

structural stage in the forward extension of the ilia and the backward displacement of the short pubis and ischia.

In the cynodonts (Figs. 6C, 7), which stood nearer to the true ancestors of the mammals, this process is even more pronounced, so that the blade of the ilium now extended well in front of the sacral articulation. By means of the latter connection the forward thrusts of the femora were transmitted to the vertebral column so that walking and running could be effected with less extreme horizontal undulation of the body and with the body well raised from the ground. At this stage, according to Romer, the iliac blade lay between the ilio-psoas and

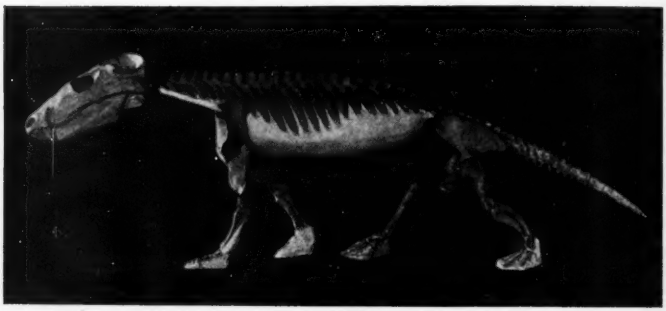

FIG. 7. Model of *Cynognathus*, based on data published by Seeley, Broom, Watson and others.

deep gluteal masses, its tuberosity serving for the attachment of the oblique abdominal muscles.

Meanwhile the pubi-ischiadic plates, which were the bases of the pubi-ischio-femoralis externus or obturator-adductor masses, have become fenestrated as the end result of a process of thinning in the middle and thickening around the periphery. Moreover, as the sacral articulation was strengthened the pubi-ischiadic plates could be reduced in size and the symphysis thinned down.

THE PELVIS OF THE EARLIER MAMMALS

Thus the pre-mammalian pelvis bears but little resemblance to the ideal mammalian pelvis excogitated by

Flower without benefit of paleontologic evidence. For the cynodont pelvis, unlike Flower's diagram, is already extremely unlike the pectoral girdle; but anatomists have often insisted on reducing the pelvis and the coracoscapular blade to a common plan, just as Vialleton, Parsons and others have imagined different ways of equating the pelvic and pectoral musculature by twisting the limbs in different directions—all arbitrary. Anatomists have also frequently relied upon the ambiguous indications of human embryology for clues as to the early history of the mammalian pelvis, and many seem reluctant even to consider the paleomorphologic evidence, still less to give it priority over assumed evidence of recapitulation in ontogeny.

Unfortunately direct paleontologic evidence as to the history of the pelvis between the cynodont and the earliest mammalian stage is lacking; however, the femur of a Jurassic mammal described by Simpson (1928, pp. 150, 151) approaches that of *Ornithorhynchus* in some features, while retaining others that are reminiscent of the higher cynodonts, so that it is not improbable that the existing monotremes may retain some correspondingly primitive features in their pelves. While there is need of a careful analysis of the very different locomotor habits of *Ornithorhynchus* and *Echidna,* with a view of discovering the underlying prototherian heritage of paleotelic characters in pelvic musculature, it seems probable at least that the marked eversion of the elbows and excessive twisting of the humerus in both genera are connected in part with fossorial habits; the same may be true of the extreme eversion of the knees and the flattening of the femora. So also the strength and thickness of certain parts of the pelvis of monotremes and the prominence of the so-called "pectineal" processes, as well as the great size of the tail (in *Ornithorhynchus*) may all be secondary; indeed Helga Pearson (1926) after a very thorough and comprehensive study concludes that most of the pelvic and thigh muscles of *Ornithorhynchus*

are essentially mammalian in their arrangement but sometimes still suggest a reptilian origin.

But even if we pass over the monotreme pelvis and compare the cynodont pelvis directly with the primitive marsupial type, we find little apparent difficulty in identifying homologous parts and in realizing the marked forward extension (Fig. 6) of the ilium and backward displacement of the pubi-ischium in the typical mammal (*cf.* Gregory and Camp, 1918, pl. XLV; Romer, 1922, pl. XLIV).

In brief, the paleomorphological evidence distinctly favors the view of van der Brock and later authors (including Straus, 1929), who regard the ilium of mammals as an essentially prismatic bone with sacral, iliac and gluteal surfaces.

The pubi-ischiadic plate of mammals was inherited directly from the mammal-like reptiles and apart from the greater development of its thyroid (obturator) fenestrae and more fully modeled borders and surfaces, it shows relatively little advance beyond the cynodont conditions.

THE PELVIS OF PRIMATES

This subject has recently been intensively studied from the mensurational view-point by Straus (1929), whose general results are in accordance with the view that the

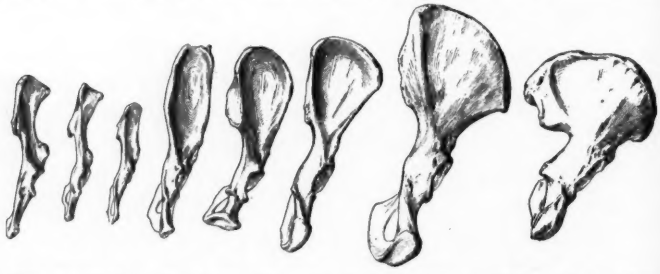

FIG. 8. Series of primate ilia, seen from the rear. Right side. After Gregory, 1920.

From left to right: *Notharctus, Lepilemur, Hapale, Alouatta, Hylobates, Pan, Gorilla, Homo.*

ilia of ancestral primates were not widely dissimilar to that of *Lemur*, and that by subsequent widening (Fig. 8) of the gluteal surfaces an ilium of the fan-shaped gorilloid type was evolved. The most primitive known primate pelvis is that of *Notharctus* (Fig. 9).

With regard to the sacral articular facets on the ilium, that of the baboon, according to Straus, is broadly U-shaped; that of man is often V-shaped but with the posterior branch longer than the anterior. In the chimpanzee and gorilla the posterior branch is very feeble and only the anterior branch remains. Therefore in effect, says Straus, the chimpanzee condition could not give rise to that of man. This is a type of reasoning frequently employed by those who set arbitrary limits to the power of nature and who rely too much both on general postulates, such as the supposed irreversibility of evolution, and on the authority of certain specialists who have assured us that man is not derived from any anthropoid ape. There is, however, an immense amount of other evidence, from many directions, that the chimpanzee and gorilla are far more closely related to man than is the baboon or any other monkey. A direct comparison of the material (Figs. 10, 11, 12) suggests that the V-shaped sacral articular facet in man results partly from the great increase in size of the sacral vertebrae, partly from the sharp angulation of the sacrum upon the lumbar vertebrae, partly from the marked secondary backward growth of the postero-superior process of the ilium. The same downward and forward pitching of the sacrum and backward and upward growth of the postero-superior process of the ilium has conditioned the sharp flexure of the upper border of the ilium at the greater sacro-sciatic notch.

The vertical shortening of the ilium of man is associated with the great widening of his gluteal and iliacus muscles and with the secondary lengthening of the loins. The conversion of long narrow ilia into wide ilia has happened independently in different phyla of mammals,

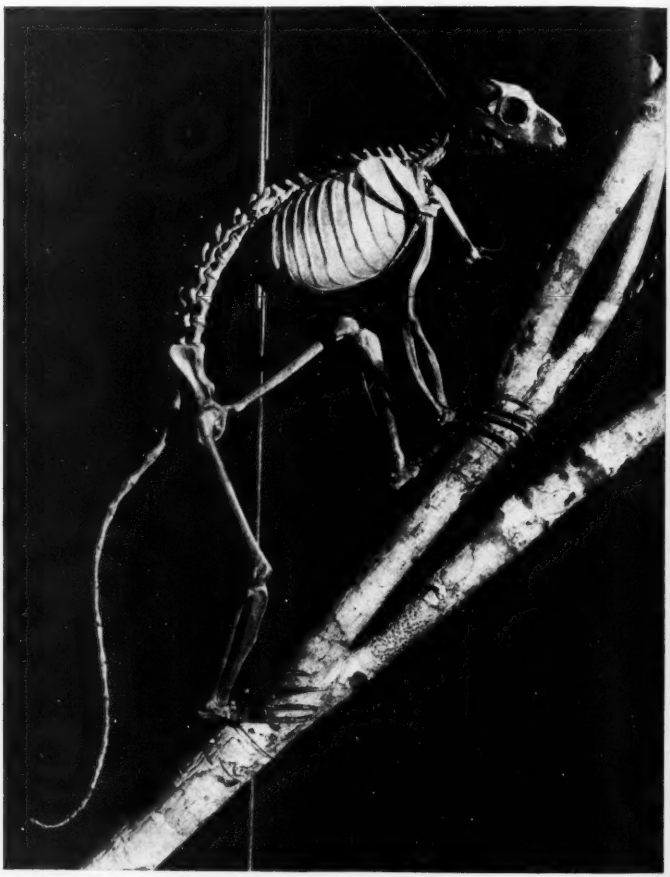

FIG. 9. Model of skeleton of *Notharctus*, an Eocene primate. Based on several incomplete skeletons in the American Museum of Natural History.

when small slender animals have been changed into large wide animals, as in the titanotheres and rhinoceroses. The vertical shortening of the human ilium, together with the dorso-posterior extension of the postero-superior portion, is a unique transformation associated with the erect position of the column, the development of the

lumbar curve and the great increase in the size of the sacral articulations.

The immense development of the anteacetabular or

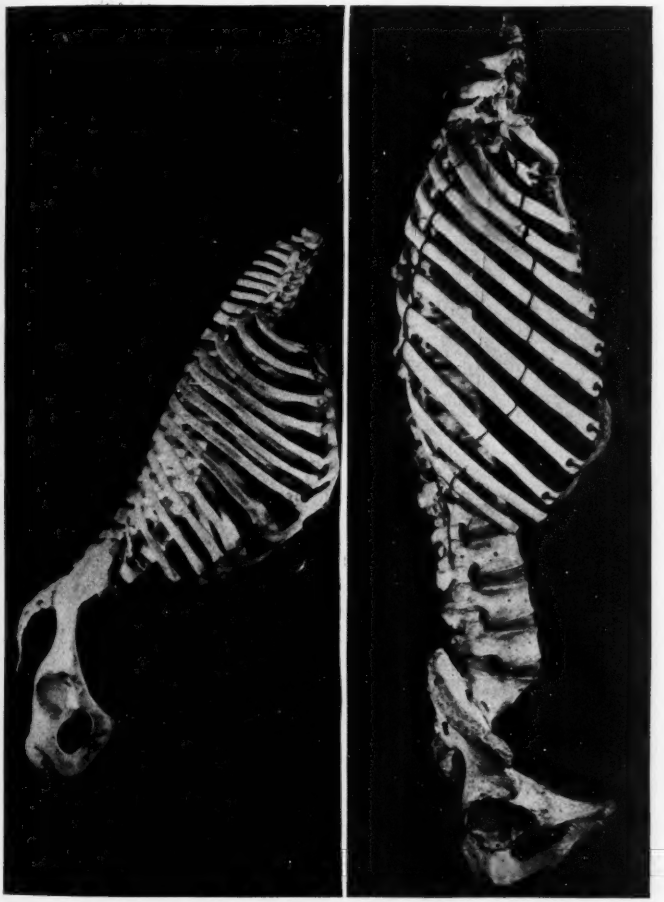

FIG. 10. Vertebral column and pelvis of chimpanzee and man, showing lumbar curve, slight in the former and extreme in the latter.

antero-inferior spine of the ilium is associated with the enlargement both of Poupart's ligament and of the tendon of the rectus femoris. Since the anteacetabular

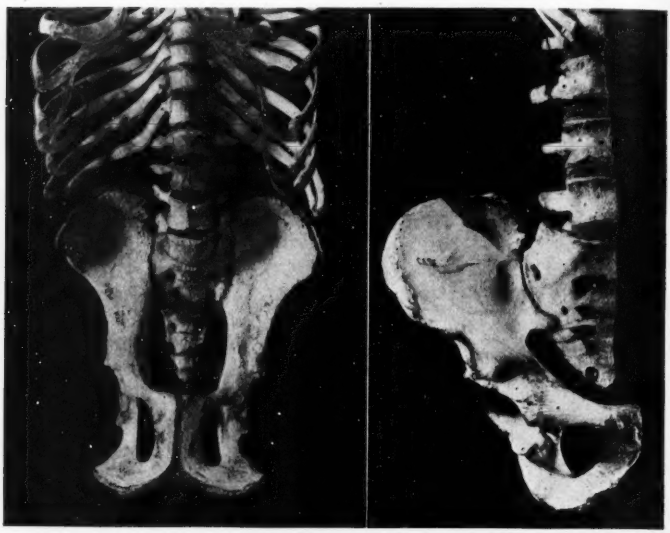

FIG. 11. Same specimens, front view, showing transverse expansion of sacrum and ilium in man.

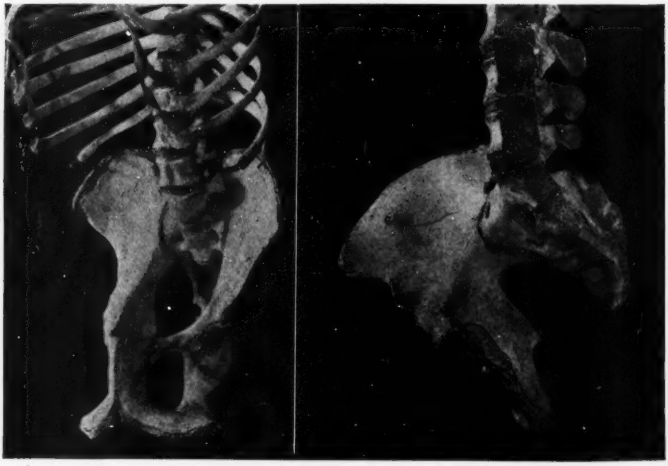

FIG. 12. Same specimens, oblique front view, showing sharp angulation of sacral facet and development of deep sacro-sciatic notch in man.

spine, which is strongly developed in lemuroids, is lost in catarrhine monkeys and but feebly developed in anthropoids, those who regard the irreversibility of evolution as infallible would again rule the anthropoids out of the line of human ascent; but Straus (1929) makes the highly probable suggestion that the great development of the anteacetabular spine in man may be a secondary adjustment to the upright gait.

Summary and Conclusions

(1) The pelvic girdle, like the pectoral girdle, arose in primitive fishes as the base of a system of lateral keels projecting from the body-wall and assisting in lateral stability.

(2) The pectoral girdle was functionally associated with the head and the branchial chamber and consisted of outer, or dermal, and inner, or endochondral, layers. The pelvic girdle was associated with the cloaca and never had any dermal sheathing bones.

(3) When the lobe-finned fishes came out on land and began to use their stout paddles as limbs, a dorsal or iliac process arose on each side from the primitive pubi-ischiadic plate.

(4) The iliac processes then grew dorsad outside of the ribs of the future sacral region; one pair of these ribs thereupon became enlarged and appressed to the medial side of the ilia.

(5) After passing through a more or less equilaterally triangular stage with the ilium immediately above the broad pubi-ischiadic plate, the ilium began to extend forward and the pubi-ischiadic plate to retreat, so that finally

(6) in early mammals the ilium appeared as a narrow triquetrous rod with faces for the spinal, iliacus and gluteal muscles.

(7) In the line of primates that led to man the ilium widened transversely, culminating in the fan-shaped ilium of the gorilla.

(8) In man the ilium has become greatly widened transversely and shortened vertically in connection with widening of the gluteal and iliacus muscles.

(9) The dorso-posterior extension of the human ilium is associated with the development of the sharp lumbar curve and with the great dorso-ventral and transverse extension of the sacral vertebrae, especially the first and second.

(10) The V-shaped iliac facet, as well as the sharp sacro-iliac notch, result from the foregoing changes.

(11) The large size of the anteacetabular spine is not a reversion to a lemuroid stage but a response to the development of Poupart's ligament and of a strong tendon of the rectus femoris.

(12) The human pelvis, like the human dentition, jaws, skull, brain, etc., represents the end term of a structural series, the penultimate terms of which are closely approximated in the corresponding parts of the modern African anthropoids. Assertions that the chimpanzee is too much specialized to give rise to man in this or that particular feature of the pelvis often rest upon wholly unproved assumptions regarding the irreversibility of evolution and are contradicted by the direct evidence of comparative osteology.

(13) The human pelvis, like many other modern osteological entities, owes its present form to the final predominance of anisomerism over polyisomerism. Arising as a result of the anisomerous enlargement and coalescence of certain skeletal rods that were the supports of an originally polyisomerous series of bilaterally paired fins, the pelvic and pectoral systems thereafter followed partly divergent, partly convergent paths on account of their respective associations either with the reproductive and eliminative, or with the acquisitive and respiratory systems. Parallel anisomerism is manifested by the dorsal growth of the ilium in the pelvis and of the scapular blade in the pectoral girdle, while secondary polyisomerism is seen in the subdivision of the bilater-

ally paired pelvic and coraco-scapular plates into three parts centering respectively around the glenoid and acetabular articulations and connected in each case by triradiate sutures. But paleontological material demonstrates that the tripartite nature of each half of the scapulo-coracoid and pelvic arches is an expression not of serial homology but of serial analogy or secondary polyisomerism. Hence

(14) Attempts to homologize the muscles and other parts of the pelvic and pectoral girdles and limbs are based on a misconception of the real nature of such correspondences as there are between them.

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FURTHER DATA ON THE INFLUENCE OF PHYSIOLOGICAL DIFFERENCES ON THE INDUCED MUTATION RATE: ANESTHESIA, STARVATION AND SEX

DR. FRANK BLAIR HANSON
BRONXVILLE, N. Y.

As has been pointed out in earlier papers, we may expect *a priori* that some changes in the physiological state of the germ cells would be accompanied by variation in the induced mutation rate. A well-known illustration of this principle is the marked difference in mutation rate found by Stadler (1928) in barley, after irradiation of dormant seeds and of sprouting seedlings. In Drosophila, an illustration is the marked decline in the lethal mutation frequency found in spermatozoa obtained at increasing intervals after irradiation (Hanson and Heys, 1929; Harris, 1929), a result which, as shown by the similar findings of Moore (1934) on visible mutations (and despite his own contrary conclusions), must be attributed mainly to differing sensitivities to the genetic effects of treatment, on the part of the different cell stages (spermatozoa, spermatogonia, etc.). Another example is the differential rate at which spermatozoa and oocytes mutate when irradiated. This fact was first noticed by Muller (1930, but mostly unpublished), but the more exact relations have been established by the present work.

It has been the aim of the present series of experiments to study the mutation frequency induced by x-rays in cells in various physiological states, and for this purpose anesthesia, starvation and aging have been combined with irradiation. The question of whether such treatments are sufficient to affect the cell so deeply as to influence the mechanism of gene mutation should have a bearing both in theoretical and practical fields. Some

reports on such work (with radium, which, however, is known to have the same genetic effects as x-rays) have already been presented by Hanson and Heys (1933 a and b, 1934 a and b). In the present series the same questions are again attacked, with the object of amplifying or correcting the conclusions previously arrived at, since there was uncertainty regarding the adequacy of that work. The justification of this repetition has appeared in the results, as these have led to conclusions some of which are considerably different from the earlier ones. It is the conviction of the author as well as of H. J. Muller, in consultation with whom the present series of experiments have been carried out, and of C. A. Offermann, who participated in the experiments, that the technique used in the present work has involved all the special precautions—physical, physiological and genetic —that were necessary, and that the conclusions thereby arrived at are quite firmly based. The present communication concerns itself with the influence of anesthesia, and with that of starvation, as well as with a comparison of the mutation rate in mature or maturing male and female germ cells.

EXPERIMENTAL SET-UP

The effect of treatment was measured by the method of counting lethals that had arisen in the X-chromosome. Where a study of the mutation rate simultaneously in males and females was aimed at, the following P_1 cross was used, instead of that of the current C1B method:

$$\frac{sc^8\ w\ (inversion)}{sc^8\ w\ (inversion)}\ \varphi \times \frac{sc^1\ delta\ 49\ (inversion)}{}\ \sigma$$

Here two chromosomes were used that had two nonlethal inversions which, together, prevented practically all crossing over in the F_1 females; these chromosomes had convenient markers for their recognition. In each case the P_1 flies for both series, the anesthetized or starved and the controls, were drawn from the same sample and x-rayed simultaneously. The F_1 virgin red-

eyed females $\frac{sc^8\ w}{sc^1\ dl\ 49}$ were crossed to sc^8 B sn w^a males in single-female cultures. The absence of red-eyed males in an F_2-culture indicates a lethal in that chromosome, *i.e.,* a lethal that was produced in the paternal treated fly, and, in the same way, the absence of white-eyed males indicates a lethal derived from the maternal treated fly. In the first series, where the rate in females was not studied, a slight modification of the above cross was used, as shown in Table I.

To produce the anesthesia during the x-raying, a simple device was used: a round glass dish with cotton and ether on the bottom, and gelatine capsules (containing the flies) on the surface, conveniently isolated from direct contact with the ether by a Cellophane disk. The concentration of the ether vapor could be regulated simply by raising or lowering the capsules in relation to the upper rim of the dish, and in this way the maximum treatment the flies could stand safely was determined and adopted. An identical set-up, except for the presence of ether, was used for the controls to the anesthetized flies, and all details of symmetry, such as periodical interchange of the positions of the capsules within the x-ray field, were carefully observed.

The starvation experiment was handled as follows. The offspring of a single pair of flies, which were derived from a strain already inbred, was divided into two lots. One, constituting the parents that were to produce the well-fed class, was transferred to food rich in yeast (6 females being allowed to lay for only 3 or 4 days in a bottle which, under more crowded conditions, would have been capable of yielding over 1,000 flies). The other lot, constituting the parents that were to produce the starved lot, was bred on ordinary syrup-maize meal medium under crowded conditions (30 females to the bottle). The offspring (F_1) of the latter batch developed so slowly that, at the same temperature (25° Centigrade), the F_2 of the fed series was collected simultaneously with the F_1 of the starved series. The relation of the weights of fed

to starved flies hatching from the above cultures was 2.2: 1. In order to increase the difference in treatment as much as possible, the metabolism of the cells in the gonads of the fed flies, on the one hand, was raised further by mating the latter, shortly after hatching and providing them with fresh yeast food, while the cell activity of the starved females, on the other hand, was lowered by keeping them as virgins on plain syrup-agar. On the third or fourth day after hatching, when the difference in the metabolic rates of the two lots was expected to be greatest (as judged in part by the rate of egg-laying), the two series were x-rayed simultaneously and subsequently bred as usual, on yeast food, for the production of offspring that were tested for lethals.

RESULTS

(*a*) *Effect of Anesthesia Treatment on Lethal Mutations:*

No differential effect on anesthetized flies as compared with the non-anesthetized controls, x-rayed at the same time, has been observed. This is seen in Table I, in which only male germ cells have been registered. Using a formula suggested by Dr. Muller to establish the most significant *weighted mean difference*[1] (*i.e.*, that with the smallest error relative to its own value), we get the following value for the series of experiments as a whole and its standard error:

Weighted mean difference between treated series and controls, $= 2.31$ *per cent.* $\pm$ *1.66 per cent.*

Attention should be called to the fact that the results

[1] The formula employed for the weighted mean difference and its error is:

$$\frac{\Sigma(w\,d)}{\Sigma w} \pm \frac{\sqrt{\Sigma(w\,p^2q^2)^2}}{\Sigma w},$$

where d = difference in lethal frequency between treated and controls in each experiment, p = proportion of lethals in treated plus controls in each experiment, q = 1 − p, and w = weight used in each experiment. The formula for the weight used is: $w = \frac{p\,q\,n_1 n_2}{n_1 + n_2}$, where n_1 and n_2 are the number of treated and of controls, respectively, in each experiment. The weighted mean frequencies themselves, and their errors, are obtainable by the use of precisely the same weights in each experiment as those used for the weighted mean difference in frequencies, the frequency, f, being merely substituted for the difference, d, in the first formula given.

agree, within the limits of error, in the separate parts of the experiment in which different lines of flies were used, and where other conditions, including the dosage, also varied. They are also in complete agreement with the results obtained by Kossikov (1934, in press) in a similar experiment with *Drosophila simulans*.

A few lethals have also been obtained in the female germ cells: 14 lethals in 289 cultures in the experimental series and 21 lethals in 456 cultures in the controls, *i.e.*, 4.8 ± 1.3 per cent. and 4.6 ± 1. per cent., respectively, the results again failing to show any significant difference.

TABLE I

MUTATION RATE IN IRRADIATED SPERMATOZOA

Series	Mating	Non-etherized lot				Etherized lot				Difference		
		No. of fertile cultures	No. of cultures with lethals	Percentage of lethals	St. error of percentage (±)	No. of fertile cultures	No. of cultures with lethals	Percentage of lethals	St. error of percentage (±)	Difference (in percentage)	St. error of difference	Ratio of difference to its error
1	X-rayed sc^8 B w^a sn ♂ mated by untreated dl 49 sc^1 virgins	765	127	16.6	1.4	674	138	20.5	1.5	+3.9	± 2.08	1.9
2	sc^1 dl 49 ♂ and virgins sc^8 w ♀, X-rayed simultaneously and mated together	456	78	17.1	1.75	289	46	15.9	2.2	−1.2	± 2.81	0.43
	Weighted mean			16.8	1.1			19.1	1.2	+2.31	± 1.66	1.4

The degree of accuracy of our present negative findings may be expressed in the statement that, if the lethal frequency was really increased by the anesthesia, it could hardly have been raised by one third, for it can be calculated from the values given, and their errors, that if an effect of the latter amount had really been produced there would have been a chance of but 1 in 50 that results as

negative as those above should have been obtained in this experiment.

(*b*) *Effect of Starvation on Lethal Mutations*

Also in this set of experiments no differential effect of treatment could be proven. As is shown in Table II, the weighted mean difference between fed and starved flies is 1.17 ± .99 for the females and 1.98 ± 1.33 for the males; in other words, the larger difference is not greater than 1½ times the standard error. The fact that these differences represent excesses on the part of the starved and not of the fed lot does not support the assumption of the gene being more susceptible to changes by external agents in cells with higher metabolic rates, where the gene, actively engaged in these cell processes, might be in a chemically less stable condition. On the basis of our results the chance of the mutation rate in the fed lot (*i.e.*, in the more actively metabolizing cells) being one tenth above that in the starved lot would fall outside of

TABLE II

Series	Starved lot				Fed lot				Difference		
	No. of fertile cultures	No. of cultures with lethals	Percentage of lethals	St. error of percentage (±)	No. of fertile cultures	No. of cultures with lethals	Percentage of lethals	St. error of percentage (±)	Difference (in percentage)	St. error of difference ±	Ratio of difference to its error
	A. Mutation Rate in Irradiated Oocytes										
1	166	20	12.1	2.2	223	14	6.2	1.9	5.8	2.9	2.0
2	348	21	6.0	1.3	462	30	6.5	1.1	−0.5	1.7	0.3
3	579	13	2.2	0.7	712	24	3.4	0.6	−0.8	0.9	0.9
Weighted mean			6.7				5.5		1.17	0.99	
	B. Mutation Rate in the Spermatozoa										
1	166	23	13.9	2.7	223	30	13.4	2.3	0.5	3.5	0.1
2	348	45	12.9	1.7	462	46	10.3	1.5	2.9	2.2	1.3
3	579	39	6.7	1.0	712	34	4.8	0.9	1.9	1.3	1.5
Weighted mean			11.0				9.1		1.98	1.33	

the range of twice the standard error; we should therefore expect it to be true in fewer than 1 case in 50 for the males, and a similar calculation gives a chance of 1 in 15 for the females. An excess of one fifth on the part of the fed lot would have only 1 chance in 1,000 for the males and of 1 in 100 for the females. On the other hand, there is only one chance in 50 that the starved males would have a mutation rate as low as 60 per cent. of that of the fed lot.

(*c*) *Differential Mutation Rate in the Mature Male Germ and the Oöcytes*

Regardless of the treatment—anesthesis or starvation—given in combination with x-rays, the male germ cells (spermatozoa) in our various experiments have shown a consistently higher mutation rate than those of the female (oöcytes), approximately in the relation of 2:1. Already in 1929 Muller had data (unpublished) which showed definitely a higher mutation frequency in males, and which taken together with his earlier results proved a significant difference, as can be seen from Table III, which he has supplied us with.

TABLE III

	In males		In females	
	No. of cult.	Lethals	No. of cult.	Lethals
(1926–27, publ. in Int. Congr. '28)	65	10	216	13
1928 (unpubl.)	260	11	260	6
1929 data unpubl. conclusion publ. 1930	402	30	324	6
Weighted mean percentage		8.30 percentage		2.85 percentage

Weighted mean difference (♂ − ♀) = 5.45 percentage ± 1.22 percentage; radio of diff./st. error = 4.45.

Shapiro and Neuhaus (1933) made comparative studies of the induced mutation rate in the second chromo-

some of *Drosophila melanogaster*. They found 20 lethals among 219 cultures for the male and 15 lethals among 204 cultures for the female, *i.e.*, 9.13 ± 1.95 per cent. and 7.35 ± 1.86 per cent., respectively. The data of the second brood there presented (12 to 24 days after treatment), which also show a difference in the same direction, are not considered here, because the lethal-containing chromosomes of the young spermatogonial cells could have multiplied after the time of raying and would be indistinguishable from independent mutations. These figures, although far too small to allow us to draw conclusions about differential rates, nevertheless point in the same direction.

The recent work of Moore on induced visible mutations shows a production of 33 in 11,620 in adult males, or .282 ± .051 per cent. (again using standard errors), and 23 in 12,525 in adult females, or .186 ± .039 per cent., the difference being .099 ± .064 per cent. Since these results, although again not by themselves sufficient for a decision, nevertheless show a difference which points as strongly as would be expected in a count of this size in the same direction as the earlier results (being well within the range of a 2:1 ratio), we can not agree with Moore that "one can safely conclude that the genic material of a cell is equally susceptible to the transmuting effects of irradiation, irrespective of the sex or stage of development . . . the results upon which this conclusion is based are in conflict with the findings of Muller (1930)."

Our own recent experiments are summarized in Table IV.

In another of our series where a dose twice as large was applied to the males, they exhibited nearly four times as many lethal mutations as the females which received the simple dose (in 744 cultures there were 124 lethals in the males and 35 lethals in the females).

All the experiments agree then in showing that the rate in the oöcytes as judged by eggs laid within a week after treatment, when kept at 26° C., is little more than

TABLE IV

Total no. of cultures (F_1–F_2)	No. of lethals in sperm	No. of lethals in oocytes	Percentage in sperm	Percentage in oocytes	Diff.
1417	144	85	10.2 ± .8	6.0 ± 0.6	+4.2 ± 1.0
1401	73	37	5.2 ± .59	2.6 ± 0.43	+2.6 ± 0.75
2818	217	122	7.7 ± .5	4.3 ± 0.38	+3.4 ± 0.62

one half that in the spermatozoa (obtained under the same conditions).

In this connection we may also mention the work by Timoféeff-Ressovsky (1931) in which he deals with the question whether a difference in apparent mutation rate may not be due to a selective process. The previous work of Harris and of Hanson and Heys left room for an alternative, as was originally pointed out by Muller, that the lower frequency of mutants observed among offspring derived from cells that had matured a longer time after the treatment was given, might have been due to an unavoidable selection of the results in the case of these cells, caused by a lower viability, or lower rate of multiplication, on the part of mutated as compared with nonmutated spermatogonia. Timoféeff-Ressovsky compares the proportion of visibles obtained from young spermatogonia and from spermatozoa with the proportion of lethals from these two stages. Since the percentage of lethal mutations falls off markedly with increasing age after treatment, whereas the percentage of visible mutations remained in his experiment apparently constant, he draws the conclusion that this postulated selective process among the growing germ cells really exists. However, his data on visibles are too small to prove this point (16 visibles among 3,300 flies for eggs laid from 1 to 15 days after treatment, and 12 among 2,900 for eggs laid from 15 to 30 days after treatment, *i.e.*, 0.47 per cent. and 0.42 per cent., respectively, with a combined error of ± 0.17 per cent.), particularly since to the error of chance variation we have to add the strong fluctuations due

to psychological factors, when visibles are looked for. The recent work of Moore, on the other hand, gives strong confirmation of the conclusion that the germ cells of the male, at different stages of maturity, really have different sensitivity to the mutational effect of irradiation, and do not undergo selective death or multiplication, although the author himself draws the contrary conclusion. In the first place, the results he reports (which are considerably larger than those of Timoféeff-Ressovsky) are on visible mutations, and they are in complete agreement with the previous results on lethals, whereas, if there had been an appreciable selective action, it should on the average have been much stronger in the case of the lethals. In the second place, his results show that the mutation rate in the X-chromosome is about the same in offspring derived from treated oogonia as in those from treated spermatogonia, whereas the opportunity for the postulated selective action would be far less in oogonia, since in them the mutations in the X-chromosome would remain in the heterozygous condition and so could exert much less effect either on the viability or on the rate of multiplication of the cells containing them. Differential mutation rates must therefore be mainly attributed to differing sensitivity of the cells to the mutational effect of irradiation.

Whereas a comparative slight difference in the rate of induction of lethals in the two sexes has been found by Shapiro and Neuhaus in the work already quoted above, a striking difference has been recorded in the same work for translocations between the second and the third chromosomes. They obtained among the offspring of treated males 30 translocations in 434 cultures, and among the offspring of treated females 1 translocation in 550 cultures, *i.e.*, 6.9 ± 1.24 per cent. and $.02 \pm .06$ per cent., respectively. In both cases the same dose was employed, and the eggs were laid from 1 to 12 days after treatment. This result falls completely outside of the range of sex difference found for the lethal mutation rate. In the production of translocations the coordinative

working of two events is required, *i.e.*, two breaks and the reattachment of the broken ends have to occur simultaneously, and in this process a new factor, space, enters, which has to be overcome. The spacial distribution of the chromosome (closely clustered together in the sperm as compared with the oöcyte) necessarily plays a part in this process.

Linking Shapiro and Neuhaus's work with our own findings, the following question arises: What relationship exists between the greater effectiveness of irradiation in raising the frequency of rearrangements in the male and in the female and its greater effectiveness in raising the frequency of "gene mutations" in the male than in the female? In other words, do the gene mutations found after irradiation represent rearrangements of chromosomal material, with resultant position effects? Special experimental work directed to this end is required for the solution of this problem.

(*d*) *Effect of Treatment on Sterility*

The sterility of the F_1–F_2 cultures in the experiment with anesthesia does not show any significant difference between the two series when an average of all the results is made (20. ± 2. per cent. for the treated as well as for the controls). Nevertheless, this value, which would show at best only the dominant sterility of the F_1 females, can hardly be considered, in this simple form, a convenient index for the determination of the genetic effectiveness of treatment, since it shows strong fluctuations due to other causes than chance variation, and these other causes are nevertheless not easy to control. The difficulties of this kind are still greater in the case of the P_1 sterility, where the fly as a whole might have been affected under the influence of the differential treatment; there is, moreover, no theoretical reason for believing that sterility of the P_1 reflects mutation frequency, since the total sterility of the fly as a whole could not depend in any simple fashion upon the frequency of the separate mutations of its individual cells. Exact results

should be given by group-tests of the fertility of various F_2 males of the same class (*i.e.*, males having the same treated X-chromosome), from the same F_2 cultures, but such tests would be too cumbersome to afford a practical index.

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SUMMARY

(1) Anesthesia did not show any significant influence on the production of mutations by x-rays.

(2) Starvation also showed no significant influence on the production of mutations by x-rays. The slight excess observed was, in fact, in favor of the starved series, a result rendering the more improbable a direct relationship between increased metabolism and a higher frequency of induced mutation.

(3) The mutation rate was found to be approximately twice as high in the spermatozoa as in the oöcytes.

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THE NON-RANDOM NATURE OF VISIBLE MUTATIONS IN DROSOPHILA

PROFESSOR WARREN P. SPENCER
COLLEGE OF WOOSTER

THE question as to whether natural mutations are random occurrences may be considered from several points of view. First, do all allelomorphs occurring at a particular genetic locus in an organism mutate at the same rate? Second, does mutation occur more frequently at some genetic loci than at others? Third, is the mutation rate higher in some races of a species than in others? Fourth, do all species mutate with the same frequency? Lastly, is the distribution of mutations in allelomorphs, loci, races and species random in time or are there periods when these are more likely to mutate than at other times? If the first four questions are answered in the affirmative, it must be concluded that mutation rate is dependent upon the innate nature of the organism. If, however, the mutation rate of several races and species varies concurrently in time it seems reasonable to conclude that certain causes of mutation are to be looked for in factors outside the organism; for it is then more likely that one causal factor in the environment might vary from time to time, possibly in a particular locality, than that inherent premutation factors should vary simultaneously in several races and species which may have come from widely separated sources geographically and genetically. It should be emphasized that internal and external causal factors of mutation may coexist.

The data presented in this study have a particular bearing on the last question raised. It seems relevant, however, to consider briefly some facts relating to the first four questions.

The work on *Drosophila melanogaster* indicates clearly that certain genetic loci mutate more frequently

than do others (Morgan, Bridges, Sturtevant, 1925). For example, mutations not artificially induced have been observed over 25 times at the white eye locus, 15 or more times each at the cut wing, rudimentary wing, vermilion eye and yellow body loci and 10 or more times in a half dozen other loci. In contrast many loci in this species are represented by a single isolated case of observed mutation. The fluctuations are not such as might be expected to occur by chance, but indicate definitely a higher mutation tendency for certain genetic loci than for others. Furthermore, it is generally these frequently mutating genes of *Drosophila melanogaster* which are paralleled by mutations observed in less extensively investigated species of Drosophila. For example, of the 15 loci in the X-chromosome of *D. melanogaster* which have mutated 4 or more times six are paralleled by mutations in the X of *D. hydei,* namely miniature, Notch, white, vermilion, scute and bobbed. In this species miniature, Notch and bobbed have been found twice or oftener.

The work of Demerec (1926, 1928, 1929a, 1929b) on the miniature allelomorphs of *D. virilis,* and of Muller (1930), Patterson (1932), Weinstein (1928), Hersh, Karrer and Loomis (1930), and Gowen and Gay (1934) on spotted or mosaic eyes in *D. melanogaster* shows that there are still other genes which are constantly mutating, some in somatic, some in germinal tissue, and some in both, the so-called ever-sporting mutants. As data continue to accumulate it becomes increasingly evident that the genetic loci in Drosophila may be divided into at least three categories, the ever-sporting loci, the frequently mutating loci, such as white eye or cut wing, and the rarely mutating loci.

An investigation of mutation rate in the several allelomorphs of a gene has naturally been attempted for loci carrying ever-sporting genes. Demerec finds that of the allelomorphs which occur at the miniature locus of *D. virilis,* the wild type and miniature 1 are stable, minia-

ture alpha mutates to wild type in both somatic and germ cells, miniature beta mutates only to the other mutable allelomorphs and not to wild type, and miniature gamma mutates to wild type only in somatic cells.

Working on white eyes, one of the frequently mutating but not ever-sporting genes, Patterson (1928) in this country and Timofeeff-Ressovsky (1929) in Germany have found that after radiation the wild type allelomorph mutates much more frequently to or toward white than does the white allelomorph to or toward red.

Perhaps the most significant work on the comparative mutation rate in two races of a species is that reported by Timofeeff-Ressovsky (1932). In an experiment involving the x-raying of an American and a Russian race of *D. melanogaster,* it was found that the American race mutated 55 times from red to white or a white allelomorph in 59,200 flies tested, while the Russian race mutated only 40 times in 75,300. The difference between the mutability of the American and the Russian stock was over three times the probable error of the difference. It was also shown that the American gene introduced into Russian chromatin retained its higher mutability. Thus races which appear alike may differ by allelomorphs which tend to mutate at varying rates.

While it is certain that the general picture of mutability may differ from species to species within a genus, further work is required before it is definitely known that the mutation rate in general in one species differs from that in another under uniform conditions. It may be concluded from the genetic evidence that mutations are not random in respect to the several allelomorphs of a gene or genes of a race or races of a species.

Let us turn now to the question of whether mutations are random occurrences in time. In a very significant report by Muller (1928) on the measurement of gene mutation rate in Drosophila, its high variability and its dependence upon temperature (a paper published subsequent to his first work on x-rays and mutation rate, but

reporting experiments done prior to and leading up to the x-ray work) it is shown that lethal mutations vary greatly in number in different experiments. In an experiment by Altenburg (Muller and Altenburg, 1919) 1 new lethal was discovered per 60 X-chromosomes tested. A second experiment by Muller and Altenburg gave 1 new lethal per 80 X-chromosomes tested. Still later in a more extensive experiment by Muller the lethal mutation rate was only about 1 per 1,000 X-chromosomes tested. The difference between the rates in the last two experiments was over 8 times its own probable error. Yet approximately one half of the X-chromosomes tested in the third experiment had come from the same genetic source as one half of the X-chromosomes tested in the second experiment. The easily controlled environmental factors, such as temperature, culture medium and vials, did not vary markedly in the two experiments. Thus there were demonstrated enormous differences of unknown origin in the lethal mutation rate in *Drosophila melanogaster* at different times.

My own work on Drosophila began in the fall of 1923 and for two years consisted of experiments on *Drosophila melanogaster,* chiefly in the synthesis and study of permutations of eye color mutants already known. During this time one case of inherited red spotting in white eyes was found (Spencer, 1926). A number of other recessive mutants were uncovered, but proved to be old ones, which were probably present in heterozygous form in the stocks received from the Columbia laboratory. The work of locating previously known mutants was good preliminary training, but that was about all.

Consequently, in November of 1925 wild Drosophila were collected from a fruit-cellar in Wooster, Ohio. There proved to be 5 species in the material, melanogaster, buskii, immigrans, hydei and funebris. Many thousands of these were raised in large gallon bottles and examined for mutations. For 3 months none were found. Then in March, 1926, a red eye appeared in the

hydei material, followed shortly by bobbed bristles and Notch wings. In May the first funebris mutant, Hairless, was found. In the course of a few more months the immigrans, buskii and melanogaster stocks were discarded, except for a few cultures of melanogaster. The latter species has never been intensively studied in my work.

The experimental cultures have been raised on standard banana agar medium, in half pint milk bottles, with cotton stoppers. From the first pedigree books, designating the number of the culture, source of parents, date and other relevant data, have been kept. In addition many of the mutants which could be carried in homozygous form have been kept in mass cultures, and these stock cultures have not received a pedigree number. However, as new stock cultures are made up from old ones the routine is to examine the flies for new mutants. The original wild stocks have been supplemented by hydei stock received from Drs. Clausen and Collins from California in 1926 and again in 1928, hydei from Dr. Sturtevant from Kansas City, Kansas, in 1927, and two infusions of new wild stock from Wooster. A number of funebris stocks descended from wild material collected in Russia and Germany have been received from Dr. N. W. Timofeeff-Ressovsky of Berlin in 1928, 1929 and 1932. Dr. R. A. Hefner has sent wild stock of melanogaster from Oxford, Ohio, in 1926. *Drosophila duncani,* descended from a single impregnated wild female, collected at Buckeye Lake in the summer of 1931, was cultured for about a year. As this species is very difficult to rear under laboratory conditions the stock died out in the summer of 1932. *Drosophila repleta,* descended from flies collected in Chicago in June, 1933, and in Canonsburg, Pennsylvania, in August, 1933, are being bred. In addition stocks of *Drosophila sulcata, Drosophila immigrans* and *Drosophila affinis* collected in Wooster in the past year have been raised in our laboratory.

It will be seen that the material has come from widely separated geographical localities. From each of these

stocks except *D. affinis* at least one mutant has been recorded. Two species, *D. hydei* and *D. funebris,* have received most attention. Only a relatively small amount of work has been done upon melanogaster, duncani, repleta, sulcata, immigrans and affinis. However, it is planned to make an intensive study of *D. repleta,* as it is a species which is taxonomically very close to hydei. According to Metz and Moses (1923) and to M. E. Metcalfe (unpublished data) the chromosome complex of these two species is very similar.

This scattering of attention on several species has, of course, the disadvantage of rather meager results in respect to mapping the chromatin of any one species. It does, however, make possible comparative studies largely free from the errors of the personal equation. Most drosophilists will agree that data secured up to the present time from various laboratories by workers with differing methods, inherent capacities and training for the observation and isolation of new mutants can not be used in comparative studies of mutation rate without the introduction of larger errors than are likely to occur in the work of one individual. As Muller has correctly pointed out, lethals are more favorable than visibles in that they tend to eliminate the subjective factor. In spite of this, an analysis of visible mutation rate seems important. It may be argued that the individual investigator changes through training, attitude towards his work and physical condition from year to year, and that these changes invalidate accurate long-time studies on mutation rate. As there is some basis for this criticism a brief analysis of these personal equation factors will be given.

My initial training in the study of eye color permutations in *Drosophila melanogaster* is reflected in the fact that of the first 12 mutations in point of time 6 were discovered through their effects on the eye, while of the total of 54 visible mutations reported in the present study only 11 were discovered through their effects on

this organ. In later years through greater familiarity with the descriptions of mutants in the literature and through an effort to concentrate attention on chaetotaxie, wing shape and venation there has been a marked shift in the proportion of eye mutants to other mutants discovered. In the course of several years' work the attitude toward variations has tended to shift as follows. At first every slight irregularity, as a few irregular facets in one eye, a crumpled wing, a crippled leg, a slight asymmetry in abdominal tergites or a bent bristle, was carefully bred through two or three generations to determine if it were a mutant. The non-genetic nature of the great majority of such asymmetrical modifications has been proved to such an extent that many such are at present recognized, but passed over with no attempt at breeding them out as time is taken with the cumulative problems on linkage and chromosome abnormalities. To offset this change in attitude toward asymmetrical modifications, a few of which might prove to be genetic, there is the ability through years of experience to inspect the ensemble of characters in each fly examined and to recognize some mutant types which in the first years might have passed unnoticed.

It might be argued that the non-mutating period from April, 1928, to May, 1931, described below represented a period of let-down following the first few years of intensive research. This was not, however, the case. In the first place, during the middle of this period, January to September, 1929, the author was in graduate school, relieved of all teaching duties and giving the major part of his time to Drosophila research. As month after month went by without the finding of mutations, smaller and less striking variations were isolated and bred from in the hope that they might prove to be mutants. It may be relevant to state that the original intent of the author was to find as many mutants as possible in species of Drosophila other than melanogaster in order to contribute to a comparative study of chromosome maps, and

the bearing of the data on mutation rate is a by-product of the original problem. Had the initial intent been a study of mutation rate per se certainly more exact records would have been kept of the actual numbers of flies in each culture examined.

The numbers of experimental cultures of which pedigree records have been kept furnish a fair though not an absolute index of the number of flies examined in the course of the work. A conservative estimate of the total number of flies would be somewhere between 750,000 and 1,000,000. In the first mutating period described below relatively more flies examined came from unnumbered large mass culture gallon bottles. Seven of the 25 mutants during this period were from this source. But throughout the work some stock cultures without pedigree numbers have been raised. The great majority of flies examined and mutants recorded have come from the numbered cultures.

After the finding of red eyes in *D. hydei* in March, 1926, mutations continued to occur for 25 months to March, 1928. In this time 25 visibles were found. Then came the depression. Month after month elapsed without the finding of a single bona fide visible mutant. That mutation could occur in this period was shown by the sporadic appearance of a sex-linked lethal in *D. hydei* and two independent somatic mutations to Notch in the same species. Studies on non-disjunction and the analysis of the material already at hand occupied the time. But the search for new visible mutations continued fruitless for a consecutive period of 38 months.

Then in June of 1931 Taxi wings, a dominant in *D. funebris,* appeared in a single male and from that time to the present 28 additional visible mutants have been found. In the first mutating period *D. funebris* and *D. hydei* each gave 11 visible mutants and *D. melanogaster* 3. This distribution represents fairly the relative amount of time devoted to each species. In the second mutating period, 11 hydei, 10 funebris, 3 duncani, 2 re-

pleta, 1 melanogaster, 1 sulcata and 1 immigrans are proportionate for all except duncani. The three mutants of this species are more than would be expected for the number of cultures raised. But the number is too small to be of significance.

The history of an inbred line of *D. funebris* is of particular interest. Ocelliless, found on July 6, 1927, is a recessive conditioning many phenotypic effects, among them complete sterility in both sexes. The mutant is therefore carried in heterozygous form by making up from 7 to 12 single brother-sister matings of normals from a culture showing ocelliless (Spencer, 1928b). In this way ocelliless has descended through the closest inbreeding for 7 years to the present through 84 generations. In generation 5, October 20, 1927, evaginated was found; in generation 11, March 27, 1928, ascute appeared. Then came the long non-mutating period followed by Taxi in generation 51, June 20, 1931, abnormal abdomen, generation 55, October 27, 1931, erect bristles, generation 64, May 16, 1932, and truncate wing, generation 71, December 17, 1932. Thus 3 mutations, ocelliless, evaginated and ascute, were found in the inbred line in the first mutating period, none in the non-mutating period, and 4, Taxi, abnormal, erect and truncate in the second mutating period. Since the origin of erect two ocelliless lines, one erect and the other not-erect, have been maintained. In the past year modifiers have developed in the erect line which suppress the expression of evaginated, while in the non-erect line these modifiers are absent. That new suppressors have occurred in the erect line has been shown by an outcross to an unrelated stock and the reappearance of evaginated in the F_2 generation. Thus the mutation history of this long inbred line, in which up to the present 84 generations and over 900 cultures have been raised, follows the same trend as that found in the remainder of the work.

In the first mutating period 1,655 pedigreed cultures were raised in 28 months. In the non-mutating period

2,343 cultures were raised in 38 months and in the second mutating period 3,591 cultures were raised in 35 months. In the first mutating period 25 visibles were recorded, of which 18 occurred in the numbered cultures. In the second mutating period 29 visibles were found of which 19 arose in the numbered cultures. Of the 54 visibles here recorded about one third have been described in previous publications (Spencer, 1927, 1928a, 1928b, 1929, 1930a, 1930b, 1932). Full descriptions of the others will not be published at this time. The dates of the discovery of the mutants, their names and the most obvious or one of the most obvious characters modified are given in Tables I and II. The dates of discovery of autosomal

TABLE I

CHRONOLOGICAL LIST OF VISIBLE MUTATIONS OF DROSOPHILA IN FIRST MUTATING PERIOD

Year	Month	Day	Name of mutant	Name of one part affected	Name of species	Found in numbered culture
1925	Nov.	.....	safranin	eye color	melanogaster	x
1926	March	3	red	eye color	hydei	
"	April	3	Notch	wings	hydei	
"	April	7	bobbed	bristles	hydei	x
"	May	10	orange	eye color	hydei	
"	May	11	Hairless	bristles	funebris	
"	July	12	Star	eyes	funebris	x
"	Aug.	3	Minute	bristles	funebris	x
"	Sept.	21	Missing	bristles	funebris	x
"	.....	.....	short	5th wing vein	melanogaster	x
"	.....	.....	triangle	wing venation	melanogaster	x
1927	Jan.	14	triangle	wing venation	hydei	x
"	Jan.	19	thickened	wing venation	hydei	
"	Feb.	13	facet	eye texture	hydei	x
"	March	10	mosaic-orange	eye color	hydei	x
"	March	14	Extra	bristles	hydei	x
"	July	6	ocelliless	bristles	funebris	x
"	July	23	interrupted	2nd cross-vein	funebris	x
"	Sept.	23	narrow	wing shape	funebris	x
"	Oct.	20	evaginated	genitalia	funebris	x
"	Nov.	10	cut	wing margin	funebris	x
1928	Feb.	.....	short	5th wing vein	funebris	x
"	March	17	squatty	body shape	hydei	
"	March	17	short	5th wing vein	hydei	
"	March	27	ascute	bristles	funebris	x

28 Months 25 Mutants 18 Mutants in the 1,655 numbered pedigree cultures

TABLE II

CHRONOLOGICAL LIST OF VISIBLE MUTATIONS OF DROSOPHILA IN SECOND MUTATING PERIOD

Year	Month	Day	Name of mutant	Name of one part affected	Name of species	Found in numbered culture
1931	June	20	Taxi	wings	funebris	x
"	July	18	Polychaete	bristles	funebris	
"	Sept.	15	miniature	wings	hydei	
"	Sept.	29	carmine	eye color	hydei	
"	Oct.	27	abnormal	abdomen	funebris	x
"	Dec.	28	Notch	wings	hydei	x
"	Dec.	28	slant	wings	duncani	x
1932	Jan.	13	vermilion	eye color	melanogaster	
"	Jan.	15	twisted	bristles	duncani	x
"	Jan.	30	cut	wing margin	duncani	x
"	March	8	tiny	bristles	funebris	x
"	March	28	cherry	eye color	hydei	x
"	May	16	erect	bristles	funebris	x
"	June	25	taxi	wings	hydei	x
"	Sept.	17	ascute	bristles	funebris	
"	Dec.	17	truncate	wings	funebris	x
"	Dec.	19	beaded	wing margin	hydei	x
1933	Jan.	14	scute	bristles	hydei	x
"	March	3	bobbed	bristles	funebris	
"	March	3	extra	bristles	funebris	
"	May	27	Duplication	wing venation	hydei	x
"	Aug.	.....	extra	wing venation	immigrans	
"	Sept.	19	spread	wings	hydei	x
"	Oct.	14	absent	bristles	hydei	
"	Nov.	25	Beaded	wing margin	funebris	x
"	Dec.	19	Extra	bristles	repleta	x
"	Dec.	20	scarlet	eye color	sulcata	
1934	April	7	irregular	hairs	hydei	x
"	April	14	eyeless	eyes	repleta	x

35 Months — 29 Mutants — 19 Mutants in the 3,591 numbered pedigree cultures

recessives are, of course, several weeks or even months subsequent to their dates of origin. Statistical analysis of the data is found in Table III. The odds against none of the 37 visibles recorded from the pedigree cultures occurring in a consecutive series of 2,343 cultures out of a total of 7,589 are 1 in 869,489. It may be safely concluded that in the period of over 8 years covered by this study the Drosophila mutations were not occurring at a constant rate in time.

As pointed out first by the author (Spencer, 1928b) and later by H. A. Timofeeff-Ressovsky (1930) and Roma-

TABLE III

PERIODIC FLUCTUATIONS IN VISIBLE MUTATIONS OF DROSOPHILA

Period	No. of months	Total mutants	No. of pedigree cultures	Mutants in pedigree cultures
1st Mutating	28	25	1,655	18
Non Mutating	38	0	2,343	0
2nd Mutating	35	29	3,591	19
Totals	101	54	7,589	37

The probability of none of the 37 visibles occurring in 2,343 chronologically consecutive cultures is $\left(\frac{5246}{7589}\right)^{37}$ or .0000011501 or 1 in 869489.

schoff and Balkaschina (1931) *D. funebris* shows certain peculiarities in the nature of its mutations when considered en masse, such as high frequency of dominants, small proportion of first-rank mutants from the standpoint of linkage study, scarcity of eye and body color mutants, etc. The phenotypic picture of mutability in *D. hydei,* on the other hand, is quite comparable to that of *D. melanogaster* with many good mutations for linkage study along with the poor ones, many eye colors, etc. Further evidence on this difference in mutability in the two species will be presented elsewhere. The fact that two species, varying as widely as do *D. funebris* and *D. hydei* not only in taxonomic characters, but in the nature of their mutations, fluctuate simultaneously in their mutation rate when kept together in the same laboratory seems to indicate that this fluctuation is not due primarily to innate premutation factors. It would be a strange coincidence indeed if the two species and various geographical races of these species used in the experiment should have developed such premutation factors simultaneously.

The evidence rather points strongly to the presence of some extrinsic factor with a tendency to fluctuate greatly at least in some localities as a causal agent in the change in mutation rate. This, of course, in no way invalidates

the argument for the presence of innate factors influencing mutation rate in different species, races, genetic loci and allelomorphic genes. In fact, the relationship between extrinsic and intrinsic factors in the production of mutations may reasonably be supposed to parallel the relationship between hereditary and environmental factors as causal agents in the conditioning of phenotypic characters. It becomes of interest to consider extrinsic factors known to condition changes in mutation rate as possible agents in the periodic fluctuations in visible mutation rate here recorded.

The classic work of Muller (1927) in demonstrating the effect of irradiation in increasing mutation frequency in *Drosophila melanogaster,* followed by corroboration and extension of this finding to other forms by workers too numerous to mention here, led to the suggestion that natural mutations may be caused by natural radiation from radio-active substances in the environment. Reports of Babcock and Collins (1929) and of Hanson and Heys (1930) on higher mutation rates in cultures of *D. melanogaster* raised where the natural radiation was high as contrasted to mutation rate in controls lent temporary credence to this suggestion. But Muller and Mott-Smith (1930) carried out careful experiments on the measurement of natural radiation and came to the conclusion that the ratio of natural mutations produced by natural radiation to all natural mutations "is probably smaller than 1 per 1000." Fluctuations in natural radiation can therefore hardly account for the fluctuations in mutation rate here recorded. Further corroborative evidence on this point is found in the results of the following experiment. In January, February and March of 1930 cultures of *D. hydei* were raised on a mixture of 1 gram of ground carnotite (a radioactive ore) to 20 grams of banana agar. Suitable sex-linked genes were used as markers and 150 single mating cultures from parents which had been reared from the egg stage on the carnotite medium were raised and examined for new sex-

linked lethals. None occurred. Nor did any visible mutations appear in the 250 F_2 cultures raised from this material. At the same time several gallon bottles of mass cultures of *D. hydei* and *D. funebris* containing carnotite were reared but no visibles were found. It was over a year after the final discarding of carnotite from the laboratory that the first visible occurred in the second mutating period. Thus the presence of carnotite in the laboratory and in rather heavy concentration in many cultures was not capable of compensating for the absence of some other factor or factors necessary to the induction of mutations during the non-mutating period.

As long ago as 1919 Muller and Altenburg demonstrated a probably significant effect of a difference between 19.5° C. and 27° C. on lethal mutation rate in *D. melanogaster*. A much more convincing experiment, using an improved genetic technique, was carried through by Muller in 1925–26 and showed a significant rise in lethal mutation rate for 27° C. over 19° C. (Muller, 1928). More recently Goldschmidt (1929) has exposed larvae of *D. melanogaster* for 24 hours to a temperature of 36° C. and reported a marked increase in mutation rate. The work of Jollos (1930) corroborated these results. Plough and Ives (1934) have repeated the temperature experiment on *D. melanogaster,* using the Goldschmidt method of heat treatment, and report a clearly significant rise in mutation rate in the over 200,000 flies in the heated lines as contrasted to the approximately 100,000 controls.

In our laboratory cultures have been raised in a basement room with temperature kept ordinarily between 23° C. and 25° C., with an occasional drop for a few hours as low as 21° C. or rise to as high as 28° C. Many of the cultures have been raised in electrically heated and thermostatically controlled incubators with a fluctuation of 1° C. or less. The optimum temperature for *D. funebris* and *D. hydei* is 24° C., and this average has been maintained throughout the 8 years of the experiment. It is certain that the mean annual temperature has not varied

over 2° C. Thus the moderate rise in mutation rate following the terrific temperature treatments of Goldschmidt and others is totally inadequate to account for the very large fluctuations in natural mutation rate found in our experiments. Plough and Ives (1934) state that, "Since Muller and Mott-Smith conclude that natural radiation is inadequate to account for mutations in nature, it seems possible to suggest that ubiquitous temperature variations may play that rôle." It seems to me, however, both from the data presented here and those reported from various laboratories on natural lethal mutation rate that neither natural radiations of short wave-length nor temperature radiations can be considered the primary extrinsic causal factors in mutation.

Summary

In a study covering over 8 years 7,589 pedigree cultures and at least 750,000 flies belonging mainly to the species funebris and hydei it was found that the rate of natural visible mutations varies greatly. During this time a non-mutating period covering 38 months and 2,343 pedigree cultures occurred. Facts are presented indicating that the cause of this fluctuation in mutation rate is neither natural radioactivity nor temperature changes. The cause or causes are, however, probably environmental rather than innate premutation factors.

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TRANSPLANTATION OF TISSUES FROM MOUSE TO RAT AND VICE VERSA[1]

DR. LEO LOEB

DEPARTMENT OF PATHOLOGY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS, MO.

IN preceding investigations, we analyzed the relations of the organismal differentials of gray Norway rats, their mutants and white and hooded rats by means of transplantations of tissues. It was of interest to compare with the reactions obtained in these transplantations those which follow transplantation of the same kinds of organs (thyroid and cartilage with the adjoining tissues, and also ovary) in two related species, such as white rat and mouse. In previous investigations, we have already studied heterotransplantations between more distant species. We were interested in these experiments also in the question, whether the results obtained in transplantations from mouse to rat would be the same as in the reciprocal transplantations. Altogether we recovered seventy-five transplanted pieces, many of which were cut into serial sections.

The following is a summary of our results.

As early as six days after transplantation, we found definite signs of degeneration or insufficient recovery in some thyroid transplants. At eight days, only exceptionally, a few thyroid acini were preserved. In one case, however, there were found even as late as twelve days after transplantation some partly degenerated acini; the ovary and striated muscle tissue also were necrotic even six days after transplantation; but occasionally some cross striation could be noticed in some muscle areas. However, cross striation could occasionally be observed in muscle fibers in which the connective

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tissue stroma separating the fibers was dead. Some connective tissue cells, lymphocytes and also polymorphonuclear leucocytes pushed their way into the necrotic muscle tissue and dissolved and partly replaced it. Bone marrow also became necrotic; it never recovered after transplantation into the new host. Gradually it was replaced by fibrillar connective tissue and masses of lymphocytes; also some polymorphonuclear leucocytes occasionally penetrated into it. The fat tissue became necrotic at an early period after transplantation. Six days after transplantation it was largely necrotic, and it likewise was gradually invaded by connective tissue cells, lymphocytes and also polymorphonuclear leucocytes; also phagocytic cells taking up small fat drops could be seen in the septa between the fat cells. It is possible that at eight or ten days some slight peripheral areas were still alive in some cases, but this was difficult to determine, on account of the marked infiltration of this tissue with other cells.

The most resistant tissues used for transplantation were cartilage and perichondrium. Even as late as twenty-five days after transplantation, the greater part of cartilage was preserved in one case. But at different periods variable results were obtained; thus in one animal there was a preservation of the greater portion of the transplanted cartilage, while in another animal of a similar character, cartilage and perichondrium soon became entirely necrotic. However, preservation of the nuclei did not necessarily signify that the metabolism of the piece was normal.

As to the mechanism which, in general, leads to the early death of these heterotransplants, it is quite evident that it is not primarily the destructive action of connective tissue cells, lymphocytes and polymorphonuclear leucocytes of the host. In some cases these factors apparently help to destroy living structures of the graft, such as acini; but it is chiefly the toxic effect of the body fluid of the host which prevents the tissues from recover-

ing after transplantation and ultimately causes their death, and these changes may take place even in the absence of active host connective tissue cells or lymphocytes. In addition, another factor seems to be of some significance in the death of the transplant. Especially in the transplanted thyroid and fat and sometimes also in the connective tissue capsule surrounding the latter, blood capillaries are seen which are very much dilated and engorged with blood; such engorgement occasionally may lead to hemorrhages or edema in the surrounding tissue. It appears as if under these unfavorable conditions, the capillaries of the host have difficulty in making connections with the transplanted vessels and thus in reestablishing a satisfactory circulation. Subsequently connective tissue and lymphocytes migrate into the various necrotic structures, dissolve and gradually replace them. Connective tissue cells and lymphocytes penetrate even into bone and cartilage and transform these tissues into soft fibrillar tissue. In some cases perhaps also living cartilage can be invaded by lymphocytes and connective tissue and be replaced by the latter. As to the relative prominence of the connective tissue cells and lymphocytes invading the different tissues, this varies in different specimens.

The injury that is inflicted on the transplanted tissues is furthermore made evident by the lack of regeneration which characterizes these heterotransplants as compared with homoio- and even with interracial transplants. Only in two specimens was there any suggestion of the possible occurrence of regenerative processes, and they were observed at as early a date as six days after transplantation, at a time therefore when perhaps the full effect of the heterotoxins had not yet been established. In one case, there was some indication of perichondrial regeneration of cartilage over a small area surrounding necrotic cartilage tissue. However, it is more probable that even in this instance we had to deal with a preformed condition in the cartilage, rather than with a real regen-

erative process in the graft. In the second case, there was a slight multiplication of muscle nuclei, indicating an abortive regeneration near the place where the muscle fibers had been injured. However, the changes were so insignificant that their interpretation remained doubtful; on the other hand, the lack of regenerative processes at later stages was quite definite.

As to the rôle of the polymorphonuclear leucocytes, this was not very significant in our experiments; in some pieces these cells were lacking altogether. When present, they were found in largest numbers at eight and ten days after transplantation and it seemed that it was especially necrotic and disintegrating fat and thyroid tissue that called forth their appearance. These tissues, under the influence of the damage inflicted upon them by heterotoxins, seem to give off substances that attract the leucocytes. The fact that the latter in some cases are especially numerous in certain portions of the transplants, suggests the possibility that localized contamination with bacteria may be a complicating factor furnishing an additional stimulus for their activity. However, such an assumption is improbable, because the technique used in our heterogenous transplantations was the same as that employed in our homoiogenous transplantations and in the latter, except during the first two or three days following transplantation, polymorphonuclear leucocytes appeared only exceptionally, and only under conditions in which a mistake in technique could be demonstrated.

On the whole, the results in the two reciprocal types of transplantation were very similar. There were, however, a few distinct differences noticeable between these two series. As a rule, the polymorphonuclear leucocytes appeared in larger numbers in the series of transplantations from rat to mouse than in the series from the mouse to rat; this is presumably due to the relatively greater amount of necrotic tissue which developed in the first series. On the other hand, fibrous tissue formation was further advanced in the mouse to rat than in

the reciprocal series, which may be accounted for by the relatively smaller size of the transplants and the smaller area of necrotic tissue that awaited organization in the mouse to rat series. Associated with this increased tendency to the formation of fibrous tissue in the mouse to rat series is the greater prominence of lymphocytic infiltration around and within these transplants. Whether this effect also is due to the smaller size of these transplants and the lesser resistance they offer to organization than the reciprocal transplants is not certain; it is rather more probable that there is a factor present in the rat which favors the marked accumulation of lymphocytes in this series. These differences between the two series were noticed at all periods following transplantation. In general—and this applies to other types of heterotransplantation as well—the accumulation of lymphocytes is greater after hetero- than after homoio- or interracial transplantations, especially in the circumference of the whole transplant, whereas in the transplanted tissues as such the lymphocytic infiltration does not need to be more pronounced.

We may state then that transplantations from mouse to rat and the reciprocal transplantations lead to much more severe injury than homoio- and even interracial transplantations; not only is the injury greater, if we consider the average results in a number of individuals, but in each individual case the injury is very severe; the great individual variations, which we have found in the case of homoio- and also of interracial transplantations, are here almost absent. We may, therefore, conclude that the individual variations in the mutual suitability of organismal differentials of mouse and rat are almost lacking. It seems then that species differentials are much more fixed and less variable than individuality and racial differentials. The only variation of importance, which we found in these heterotransplantations between rat and mouse, concerns the length of time during which cartilage and perichondrium are preserved and this variation is probably more apparent than real, inasmuch

as even in those cases in which these tissues seemed to be preserved they did in all probability not function normally, as evidenced by their lack of regenerative power.

But we must also consider the possibility that this relative uniformity in the intensity of reaction of the host against the strange transplants is due to the fact that the possible limit of the severity of reaction has almost been reached in these cases, and that therefore considerable variations in individual instances are impossible. In general, the usual reaction against these heterotransplants corresponds to the most severe reaction which occurs after homoio- or interracial transplantation.

CONCLUSION

The reaction of the host against the grafted tissue in mouse to rat or rat to mouse transplantations corresponds to the most severe reaction observed in homoio- or interracial transplantations. The considerable variations in the results in individual cases which we observed in the latter kinds of transplantation is lacking after heterotransplantation between mouse and rat; here variations are almost limited to the length of time during which preservation of cartilage and perichondrium is observed. Regenerative processes are entirely or almost entirely lacking in these heterogenous transplants. The destruction of the graft is largely due to the unfavorable effect of the body fluids of the host, which does not permit the establishment of a satisfactory circulation around the transplant at an early period following transplantation and may act also otherwise as toxic substances. The uniformity in the results and the lack of individual variations noted in transplantations between rat and mouse is presumably due to a relative lack of variability in the species differentials, in contrast with the considerable variations which are found between individuality differentials. We must also consider the fact that the limit in the severity of the reaction has nearly been reached in the transplantation between mouse and rat.

EVIDENCE FOR THE PROTECTIVE VALUE OF CHANGEABLE COLORATION IN FISHES

DR. FRANCIS B. SUMNER

SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA

FOR reasons which are not difficult to see, the related subjects of protective coloration and mimicry have played a prominent part in biological history since the days of Darwin and Wallace. For equally obvious reasons, we frequently find these topics looked upon with suspicion by some of our more critical recent biologists. Much of the literature in this field is merely descriptive. Examples of these phenomena are described in detail, no evidence for the prevailing biological interpretation being regarded as necessary. In the course of such studies, many striking and indeed startling resemblances have doubtless been brought to light. On the other hand, much unbridled speculation has been indulged in. Not infrequently, high significance has been attached to what are with little doubt purely accidental correspondences. And again, animals which would seem to be conspicuously colored in any possible natural environment are offered to us as being, in reality, fine examples of concealing coloration. The dogma has even been promulgated that the colors of all animals are of concealing value, if viewed under conditions where concealment is necessary. Thus, Abbott Thayer paints his flamingoes against a lurid sunset sky, while he so poses a peacock that we view its green tail feathers against a background of foliage, while the head and neck are seen against a conveniently handy patch of blue sky (Thayer, 1909).[1] It is hardly surprising that some biologists have indignantly repudiated the whole notion of protective coloration.

There is, however, a considerable literature dealing with experimental tests of the protective value of animal coloration. The results of such experiments, conducted

[1] We must not, on this account, overlook the high value of some of Thayer's demonstrations of the optical principles underlying concealment.

chiefly upon insects, have not been at all in accord with one another. So far as they have dealt with the value of supposedly "warning" coloration, they are not relevant to the subject-matter of the present paper. And, indeed, scarcely any of them have been concerned with the phenomena of adaptive color *change,* which is my present topic of discussion.

It is well known that whereas most concealingly colored animals display a fixed color scheme which is adjusted to some more usual habitat of the organism ("fixed homochromy"), many are endowed with the faculty of changing their colors and bringing them into conformity with different surroundings ("changeable homochromy").

It happens that the voluminous literature dealing with the functional value of homochromy relates almost entirely to the first of these categories, the fixed type. It seldom concerns itself with the functional value of changeable homochromy. It is true that the mechanism responsible for these color changes has been studied intensively for half a century and described in great detail. But the biological significance of this widely distributed mechanism has either been tacitly assumed, or has been made the subject of more or less academic discussions. I am aware of only one previous case in which the biological utility of this mechanism has been subjected to experimental test.

And yet the mechanism is in many cases a thing of such extraordinary complexity that we can not conceive of its being evolved independently in several distinct branches of the animal kingdom unless it rendered some highly important functional service.

It is the object of the present paper to consider the biological significance of the chromatic function, particularly in one group of animals, the fishes.

Before proceeding, it is well to recall that the effector organs (color-cells or chromatophores) responsible for these color changes in fishes are of several types. The most widely prevalent, and by far the most carefully studied, are the black chromatophores, the melanophores.

But there are also white chromatophores (guanophores), and at least two kinds of truly colored ones (xanthophores and erythrophores). In all these the visible responses to stimuli (or, at least, the rapid ones) result from the movements of the pigment granules within the cells. When, for example, the melanin granules of the melanophores are dispersed throughout the entire cell-bodies the fish becomes dark; when they are concentrated into small specks at the center of each cell, the fish becomes pale. Similarly with the colored chromatophores.

We thus have both changes of color proper and changes of shade (from light to dark or *vice versa*). In reality, the changes of shade, resulting from the dispersion or concentration of melanin, are of more wide-spread occurrence or, at least, are far more in evidence than changes of color *sensu stricto*. Yet, owing to the poverty of our language, we seem condemned to class all these phenomena as "*color* change," and to speak of black and white "*chroma*tophores."

It is obvious that changes of shade may be perceived quite independently of any power of color vision. And since most of the "color" changes undergone by fishes are largely or wholly changes in shade, the ready argument of the critic who challenges us to prove that the enemies of fishes have color vision may be ignored.

To proceed, then, with our interpretation of the biological significance of "color change" in fishes. As will appear in the ensuing pages, my own interpretation is the obvious, indeed the "orthodox" one, namely, that the primary object of these changes is concealment, either from the animals' predaceous enemies or from their own prey. In support of this contention (at least as regards escape from predators), I am submitting a body of experimental evidence recently obtained by me.

Before doing so, however, it may be worth while to consider some of the objections which have been raised against the concealing coloration hypothesis in general,[2]

[2] *I.e.*, the hypothesis that concealment is the real "object" of this function, not merely an incidental result.

and some of the alternative hypotheses which have been offered in its place. Most of these objections were originally applied, to be sure, to cases of fixed homochromy. But, if true, they would equally affect our interpretation of changeable homochromy. Some of these objections may have a limited, though by no means a general, application. Others seem altogether frivolous.

(1) The resemblances are the result of chance coincidence. With animals displaying such an endless variety of color and form, it would be surprising if resemblances to surrounding objects did not occasionally occur. One would hardly dispute the occasional validity of this contention. Doubtless, some of the supposed cases of "protective resemblance" are purely accidental and of no value to the animals concerned. However, we may cite cases where a considerable part of the entire fauna of a given habitat (*e.g.,* the *Sargassum*) has taken on the color scheme of the latter. Many cases are also known in which detailed resemblances in both form and markings have been adopted.

(2) The very fact last mentioned, *i.e.,* the occurrence of such extraordinarily detailed correspondences, has been cited as an argument *against* their protective significance. The camouflage would seem to have been overdone. A bird or other predator would be misled by much less of a disguise. However, we do not know enough about the visual discrimination of predators to make such sweeping assertions. Some experimenters, indeed, have found that birds are able to see through some disguises, and have concluded accordingly that these afford no protection at all (Cuénot, 1911). As regards those extraordinarily detailed disguises, which fill us with such amazement, the only alternatives to the conventional hypothesis of "protective resemblance" would seem to impose a far greater burden upon our imagination. I can only think of three: "chance" coincidence, special creation or the assumption of some incredible propensity on the part of organisms to copy what they see about them.

Does it not strain our credulity less to believe that the predator in question is so sharp-sighted that a less complete disguise on the part of its prey would be insufficient? (We may, of course, suit this argument to the disguise of the predator from its prey.)

(3) Direct physical or chemical causes may be assigned in many cases for homochromy, without appeal to the need for protective coloration. For example, many animals obtain their pigments directly from the plants or animals on which they feed. Granted, but the fact is not especially relevant. One of the most extreme exploiters of the protective coloration idea (Poulton) was among the first to point out the direct derivation of pigments from food.

(4) Animals which are well concealed on certain backgrounds do not restrict themselves to these backgrounds. Why should we expect them to? No adaptation, morphological or physiological, is ever perfect.

(5) If homochromy is so important, why do not all animals display it? And why do animals which are not concealed seem to fare as well as those which are? One would need to consider each case upon its merits. Different animals have very different enemies and very different conditions of life. Homochromy is only one of many adaptations favoring survival.

(6) Predacious animals are actually known to eat many supposedly protected forms. This argument, which is merely another aspect of the last, has recently been made much of by McAtee (1932), who bases his beliefs upon an examination of the stomach contents of many thousands of birds. He concludes that "availability undoubtedly is the chief factor involved in the choice of food, and predation therefore tends to be in proportion to population" (*i.e.*, the population of each group of food organisms present in any given locality). "Considering bird predation alone this principle leads to a high degree of indiscriminacy in attack upon the whole kingdom of animal life. . . . And this is only another way of saying

that the phenomena classed by theorists as protective adaptations have little or no effectiveness." McAtee's argument, which he seems to apply to all supposedly protective adaptations whatever, has been criticized by a number of recent writers.[3] Were he content with the first rather general assertion quoted above, it is likely that few would dissent from his position. But the last sentence appears to be distinctly a *non sequitur*. Granted that availability is an important, or even the chief factor, in the acquisition of food, there remains the likelihood of a certain amount of selection on other grounds. And continued selection, even if statistically of low intensity, may achieve very considerable results. That, in reality, birds do select the more conspicuous individuals among fishes which are equally "available" in the spatial sense is shown conclusively by my own recent experiments.

(7) Many animals do not have color vision. This argument has already been referred to. Many animals are now known which do have color vision, including some which had been supposed to lack it. In any event, this argument does not apply to cases of conformity in *shade* (*i.e.*, paleness or darkness), which is even more familiar than conformity in *color*, properly speaking.

(8) The whole theory of "protective coloration" is "anthropomorphic," "teleological," "finalistic" and not really an explanation at all.[4] Were it not that several biologists of prominence have been emphatic in making, and reiterating, assertions of this sort, it would hardly seem necessary to refer to them. Such methods of argumentation would seem to belong rather to political oratory than to sober scientific discussion. Scientific theories are seldom demolished by the use of epithets.

"Just why it is unscientific or anthropomorphic to hold that a black animal against a black background is less likely to be eaten by a bird or a fish than if it were white, or that its present capacity for self-concealment has re-

[3] Poulton, Huxley, Nicholson, Cott (all 1932); Benson (1933).

[4] *Cf.* Fuchs, 1913, 1914; Verne, 1926; Cuénot, 1927.

sulted from the survival of those strains in which this faculty was best developed, is not easy to understand.'"[5] We do not commonly attach any teleological significance to the operations of a sieve! The only question for the scientist to decide is whether such a sieve exists. In the present case, we have abundant evidence that it does.

Restricting ourselves henceforth to changeable homochromy—to the adjustments of animals to the colors or shades of a varying environment—I am aware of only two alternatives to the "protective coloration" hypothesis which have been offered. Neither of these can have, at best, more than a very limited application.

(1) Cuénot's "antispectral" hypothesis (Cuénot, 1927), which he himself has only applied to certain shrimps that dwell among variously colored sea-weed and adjust their colors to those of the weed. These shrimps are negatively phototropic, from which Cuénot concludes that they are unfavorably affected by light. His explanation is rather ingenious. An individual dwelling in green sea-weed, for example, receives upon its surface chiefly green light, transmitted or reflected by the plant. In order to exclude this light from the body, the integument of the animal likewise turns green, thus reflecting at the surface those wave-lengths which succeeded in passing through the green filter. Query: would it not have been much simpler for the shrimp to acquire an opaque integument?

(2) The hypothesis, originating with Max Weber, that some, at least, of the color changes of animals enable them to absorb or reflect heat-rays. This zoologist pointed out that in vertebrates chromatophores are chiefly found among poikilotherms. Thus far, I have made no reference to the occurrence of color changes other than those called forth by the background. It is well known that such changes are induced by a considerable variety of stimuli, both chemical and physical. Thus certain amphibia and reptiles are known to become

[5] Sumner, 1934, p. 77.

dark when their temperature falls below a certain level, becoming pale again when this temperature is reached or exceeded. Such a mechanism would be useful in enabling the animal to absorb the sun's rays when needed and to reflect them when they might be harmful.

Fuchs (1913, 1914) has adopted this hypothesis and attempted to apply it to all animals capable of color change by means of chromatophores. (May we not apply to these the term "poikilochromic?"). He is not content to explain in this way the known color responses of various land animals to temperature. Here, there would seem to be an actual, though limited, application of the hypothesis.[6] But Fuchs endeavors to extend this explanation to those color changes which bring the animal into conformity with its background. A dark animal on a pale background, he claims, would tend to become overheated; while a pale one on a dark background would have heat drawn away from it.

Passing by the improbability of this contention, for which no evidence is offered, it has already been pointed out by Bauer (1914) and Buddenbrock (1928) that a fish, under water, could not accumulate an appreciable amount of solar radiation by turning black. And yet, in spite of these fundamental difficulties, Fuchs offered his hypothesis as being "purely physiological," instead of "anthropomorphic"!

Finally, we must remember that no such theory can explain the adaptive changes of *pattern,* which are displayed in a marvelous manner by certain bottom-dwelling fishes. These changes can have no other object than concealment. Though described in detail more than twenty years ago (Sumner, 1911; Mast, 1916), these phenomena have been persistently overlooked by the opponents of the protective coloration hypothesis.

This whole situation is decidedly one in which experimentation is called for. Although numerous experiments have been performed to test the protective value

[6] Redfield (1918) has described a striking case of this sort in the "horned toad" (*Phrynosoma*).

of fixed homochromy (chiefly on insects), I know of but one previous case in which the animals concerned were ones having the power of color change.

Cuénot (1927) performed an apparently quite limited number of experiments in which certain shrimps were exposed in aquaria to the attacks of fishes. The shrimps were species whose colors were ordinarily closely adjusted to the particular sea-weeds on which they happened to be or to the bottoms on which they lay. Cuénot placed them, either living or dead (pinned), partly upon sea-weeds of their own color, partly upon ones of contrasting colors, partly upon sand.[7] Some specimens of a transparent species were injected with intra-vitam stains. From these tests Cuénot concludes that a fish is as likely to catch and devour a shrimp which is not adjusted to its background as one which is. He believes that, regardless of color, these crustacea are protected from fishes so long as they remain motionless. As soon as they move, they are likely to be pursued and caught.

The data which Cuénot offers are in no sense statistical. It seems quite doubtful from his account of the experiments whether a moderate degree of selection, such as appears, for example, in the first half of my Table 1, would have been detected.

In my own experiments small fishes were employed as prey. The species used was in every case the familiar mosquito fish (*Gambusia patruelis* (Baird and Girard)). Previous to the experiments, the fishes had been "conditioned" by keeping them for some weeks (31 to 58 days) in large laboratory tanks, the inner surfaces of which had been painted black and white, respectively. It is now known that a prolonged sojourn under these conditions results in a great increase in melanin pigment, in the one case, and a corresponding loss in the other.[8] When fishes of these contrasting histories are placed

[7] The species chiefly used was one in which the power of color change is largely restricted to its earlier life. But the animals, when left to themselves, more frequently seek out weeds of their own color.

[8] *Cf.* Sumner and Wells (1933).

together upon the same background, there is a rather rapid partial approximation of their shades, due to changes in the aggregation of the melanin in the chromatophores. But the two lots remain distinguishable for some days (Figs. 1 to 4).

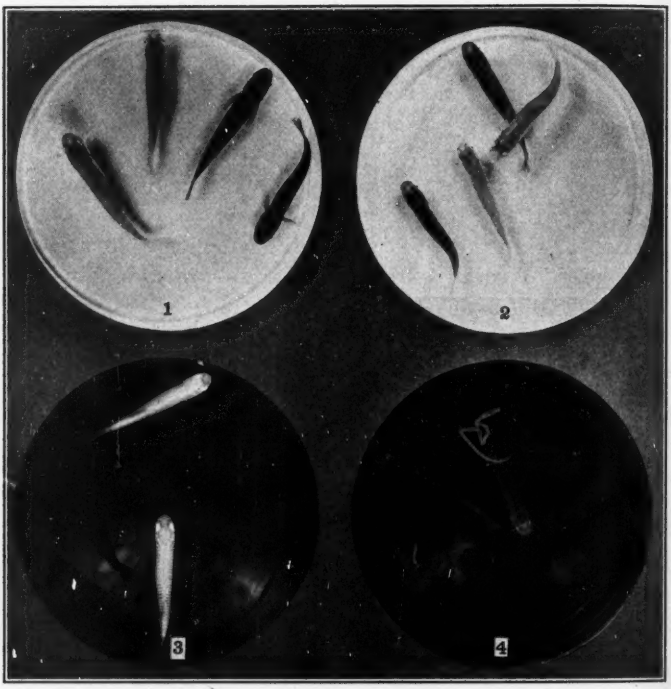

FIG. 1. Four specimens of *Gambusia patruelis*, two of which had been kept in a black tank for about seven weeks, the other two having been kept in a white tank for an equal period. Photographed on a pale gray background, fifteen seconds after transfer of the black fishes.

FIG. 2. The same fishes after remaining for 24 hours upon the pale gray background.

FIG. 3. Four specimens of *Gambusia*, of the same history of those in Fig. 1, but taken 20 seconds after transfer of the white fishes to a black dish. (The "white" fishes appear too pale in this photograph).

FIG. 4. The same fishes after remaining for 27 hours in the black dish.

For the purposes of these experiments, two large wooden tanks were constructed having internal dimen-

sions of $15 \times 8 \times 3$ feet, the last figure representing the depth. One of these tanks was painted black within, the other a very pale gray, somewhere between Ridgway's "pale olive-gray" and "light olive-gray."[9]

In the various experiments to be described, the two lots of fishes (black and white) were poured into a given tank, at the same time and in equal numbers. Up to that moment, each lot was kept in the black or white can in which it was transported from the laboratory. The fishes were admitted to the tanks, either simultaneously with the birds or before or after these, depending upon the experiment.

At the conclusion of each test, the fishes were removed and counted. In all the later experiments a separate count was made of those which had been injured but not eaten. Care was taken to remove every fish from the tanks by use of nets or otherwise. The identification of the fishes as "blacks" or "whites," even after a sojourn of many hours in a common tank, presented little difficulty, though it frequently required time. For this purpose, black and white bowls were employed. Such few specimens as could not be identified with certainty at a glance could be distinguished beyond doubt by placing them upon a background other than that from which they had immediately been taken. A "black" fish, for example, taken from the pale tank, could be speedily restored to full black by transfer to a black bowl. A "white" fish, under these conditions, would remain conspicuously ill-adjusted.[10] Thus, with the exception of two specimens in Table 1, and, for special reasons, many of those in the experiment with black bass, no serious doubt was felt in any individual case. My own identifi-

[9] This shade was chosen as being a closer approach to that of the "white" fishes than pure white would have been. The tanks were cleaned from time to time, and the bottoms kept free from falling leaves or other debris. That of the gray tank became stained somewhat yellowish in places, but romained very pale.

[10] Urethane also proved useful in some cases, as stated in my earlier report, but its use was found to be unnecessary.

cations were confirmed by Dr. Wells on the occasions when he participated in making the counts.

These experiments were conducted at the San Diego Zoological Gardens and their success would have been quite impossible without the cooperation of members of the staff of that institution. My acknowledgment and hearty thanks are due to Mrs. Belle J. Benchley, the director of the "Zoo," who placed these facilities at my disposal, and to Messrs. Karl Koch and Bayard Cihlar, who rendered important assistance throughout. I must also record the valuable help rendered by Dr. N. A. Wells, of the staff of the Scripps Institution, and by my son, Herbert Sumner, who participated in some of the experiments. To the officers of the Steinhart Aquarium of the California Academy of Sciences I am indebted for several thousand *Gambusia,* which were caught and brought to La Jolla on two occasions by Mr. W. R. Martin.

The first experiments were conducted with the Galapagos penguin (*Spheniscus mendiculus* Sundevall). This bird was chosen, partly because of its availability, partly because of its keen appetite for fishes and complete indifference to the presence of observers. Two birds were used in each experiment, but the number of different individuals used in the various tests was at least four and probably considerably more than this. The specimens in question were caught at the Galapagos Islands and had all been at the "Zoo" for at least a year. During that entire period, their diet had consisted of dead fish. They never had an opportunity to catch living ones. This fact did not, however, affect their prompt and successful pursuit of the *Gambusia.*

This bird catches its prey by swimming under water, using its diminutive wings as organs of propulsion. In our experiments, the presence of the fishes was apparently not detected until the bird's head was thrust below the surface of the water. After admission of the birds, pursuit of the prey commenced within a period ranging from a few seconds to several minutes. Thereafter, the

pursuit was fairly continuous, interrupted by the birds' coming up for air. The water was kept from two to two and a half feet deep. The experiments were conducted when the sun was fairly high in the sky and the tanks, though shaded somewhat by trees, were well lighted.

Table 1 itemizes the results for the penguin experiments.[11]

It will be seen that 1,726 fishes were employed in this series, 1,056 in the pale tank, 670 in the black. Of these, 749, or about 43 per cent., were eaten by the birds. Almost identical percentages of the total number were eaten in the two tanks, although the average time allowed the birds was considerably greater in the pale tank.

The significant fact is the very different proportion of "black" and "white" fishes eaten in the two tanks. In the pale tank, 278 fishes, or 61 per cent. of those eaten, were "blacks," while 176, or 39 per cent., were "whites." In the black tank, on the contrary, only 78 fishes, or 26 per cent., were "blacks," while 217, or 74 per cent., were whites." Moreover, in every single instance, among the 11 experiments here comprised, the trend of the results for the same tank was the same.

It is not surprising that the difference between the percentages of "blacks" and "whites" eaten (*i.e.*, the intensity of the selection) was much greater in the black tank than in the pale one. To the human observer, the fishes of both classes in the pale tank were readily visible, though not equally conspicuous. In the black tank, the "black" fishes were scarcely visible at all, except when seen at short range, or in particularly favorable illumination. The "whites," on the other hand, could be readily seen from a considerable distance.

But there is one phase of these results which was quite unexpected. In both of the tanks there is as much evidence of selection when the fishes had been left there for nearly or quite a day before the admission of the birds

[11] These figures, with the exception of those for October 5 and 9, have already been published (Sumner, 1934a).

TABLE 1
EXPERIMENTS WITH PENGUINS

	Date 1934	Serial no. of exper.	No. of fishes used	Interval between admitting fishes and birds	Time allowed birds in tank[1]	Eaten		Injured		
						black	white	black	white	doubtful
Pale tank	Aug. 3	1	100	none	2 min.+	14	6			
		2	100	none	4′ 10″	29	14			
		3	76	none	4 min.	22	19			
	Aug. 9	8	300	11 min.	6 min.+	100	64			2
	Oct. 5	19	200	20 hrs.	15 min.	20	10	5	4	
	Oct. 9	20	280	27 hrs.	60 min.	93	63	9	5	
	Totals		1056			278	176	14	9	2
	Percentages of those eaten					61%	39%			
Black tank	Aug. 6	4	90	none	4 min.	13	34			
		5	90	none	5 min.+	17	43			
		6	90	5 min.	5 min.	17	42			
	Aug. 9	7	200	10 min.	8–9 min.	26	82			
	Oct. 5	18	200	20 hrs.	9 min.	5	16	4	2	
	Totals		670			78	217			
	Percentages of those eaten					26%	74%			

[1] Recorded from commencement of pursuit of fishes.

(experiments 18, 19, 20) as when the fishes and birds were admitted simultaneously or nearly so (experiments 1–8). Indeed, the percentages in the two cases are closely similar, in the pale tank almost identical. From Figs. 2 and 4 it is evident that a considerable degree of approximation in shade, on the part of the two lots of fishes, takes place in the course of 24 hours. However, it must be pointed out that the degree of visible difference among the prey can not be the only factor determining the intensity of selection in such cases as these. The keenness of sight, degree of hunger and other circumstances relating to the predators must play an important part in the result. In the absence of any evidence, we may regard it as highly probable that the same individual birds, in the same state of hunger, would display an intensity of selection which would be higher in proportion to the visible difference among the fishes.

The question whether there may not be differences in the relative activity of our two lots of fishes will be discussed after the next series of experiments has been considered.

In only the later penguin experiments (18, 19, 20) was a careful count made of the fishes which were injured by the birds but not eaten. Since it was not always possible to detect slight injuries, the count was here restricted to fishes which bore unmistakable evidence of having been seized. In Table 1, the data from injured fishes are meager and not even consistent. They will be found much more impressive when Table 2 is examined.

In addition to a diving bird such as the penguin, it was thought desirable to employ one of the waders. For this purpose we used a night heron from Cocos Island (species undetermined). This bird was likewise chosen largely because of its availability,[12] but it proved to be an admirable performer. The specimen used had been in

[12] It was found difficult to trap these birds in the large flying cage at the "Zoo," and we consequently contented ourselves with this single very satisfactory specimen.

captivity at San Diego for three or more years, during which time it never had access to living fish.

As is well known, the feeding habits of the wading birds are very different from those of the divers. The former stand in shallow water, moving about slowly from point to point in search of prey, but striking with great rapidity when a fish falls within range. The birds are relatively tall, and the fishes are seen from some distance above the water. In these experiments the depth of the water in the tanks was reduced to two or three inches. In view of the flying powers of the heron, it was necessary to cover each tank, when in use, by a roof of netting.

The results of the heron experiments are presented in Table 2. *This series yields as indubitable evidence as the earlier one for the selection of the more conspicuous fishes.* The great differences in the numbers consumed in the several experiments seem to relate in part to the duration of the latter, while the degree of discrimination may have depended upon the time of day when most of the feeding was done. Thus, the three hours covered by experiment 13, in the pale tank, were in the middle of the day. No discrimination whatever is to be noted in the case of the few fishes which were eaten, though the numbers of injured were 10 "blacks" to 6 "whites." In experiment 17, the fishes were placed in the same tank at dusk, and the survivors recovered rather early on the following morning. It is probable that most of the feeding was done during the twilight, if not actually during the night. This experiment yielded the greatest evidence of selection in the entire series. It may be significant that the next highest degree of selection was shown in experiment 16, in which the fishes were also introduced into the tank (this time the black one) late in the afternoon and were left through the night.

It would be difficult to explain why the intensity of selection, as shown by the gross percentages, was no higher (indeed was actually slightly lower) in the black tank than in the pale one. However, as has already been

TABLE 2
EXPERIMENTS WITH NIGHT HERON

	Date 1934	Serial no. of exper.	No. of fishes used	Time allowed birds for feeding	Beginning and end of experiment	Eaten		Injured	
						black	white	black	white
Pale tank	Sept. 26	13	200	3 hrs. ±	10:30 a.m. to 1:30 p.m.	11	11	10	6
	Oct. 3	17	200	14 hr. +	5:45 p.m. to 8:00 a.m.	40	19	6	5
	Totals		400			51	30	16	11
	Percentages of those eaten					63%	37%		
Black tank	Sept. 28	14	200	23 hrs. ±	10:30 a.m. to 9:30 a.m.	22	37	5	7
	Sept. 29	15	200	22 hrs. ±	11 a.m. to 9 a.m.	42	52	4	7
	Oct. 2	16	200	16 hrs. ±	4:40 p.m. to 8:45 a.m.	21	40	5	13
	Totals		600			85	129	14	27
	Percentages of those eaten					40%	60%		

pointed out, the various experiments of this series are hardly comparable with one another, owing to the inequality of conditions in the different cases.

Despite small numbers, the figures for injured fishes point in the same direction as those for the fishes which were eaten.

Thus, for both the penguins (divers) and the heron (a wader), we have conclusive statistical evidence for the predominant selection by the birds of those fishes which were least in harmony with their backgrounds. In the case of the penguins, the evidence for selection on this basis was equally decisive when the two lots of fishes had been kept together for a day upon a common background, and the original differences thus greatly reduced.

It may be pointed out here that the restricted quarters in which these experiments were conducted would tend to make for a more indiscriminate capture of the fishes than would ordinarily occur in nature, since predators and prey were forced into greater propinquity than would be likely in the open. This gives the positive results yielded by these tests an even higher significance.

The possibility of another interpretation of these results early suggested itself to us, as it may well have done to the reader. Might not the selective elimination of fishes of one shade be due not to differences of appearance but to differences in activity? I have already discussed this possibility in my preliminary report upon these experiments (Sumner, 1934a), and admitted the possibility that a higher degree of activity on the part of the "white" fishes may have been accountable in part for their lower rate of mortality in the pale tank. By the same token, however, the differential mortality in the black tank would be even more significant than the figures appear to show.

I now know that I conceded altogether too much possibility to this suggestion. It is true that in small aquaria in the laboratory "white" fishes in white aquaria are more active or "timid" than black fishes in

black aquaria. And it is likewise true that black fishes retain this lower degree of activity when transferred to a white container, even after the lapse of an hour or more. But these differences in activity gradually disappear and are probably not observable after 24 hours.

The following facts would seem to dispose completely, however, of this interpretation of any aspect of our selection results: (1) At the commencement of experiments 19 and 20, observations were made of the behavior of the fishes when poured into the pale tank. The "black" and "white" lots promptly united into a single school and, until disturbed, swam together as a unit. I repeatedly tried the experiment of breaking up the school by dipping a long stick into its midst. Small detachments swam in every direction, but there was no preponderant tendency for the "white" fishes to be in the lead on such occasions. The two lots behaved exactly alike.

(2) In experiments 18, 19 and 20, the fishes were introduced into the tanks nearly or quite a day before the birds were admitted. There was nevertheless equally conclusive evidence of selection.

Thus, it is clear that in neither tank has the differential mortality of the "black" and "white" fishes been due to differences in their ability to outdistance their pursuers.

In all these experiments, to be sure, the difference between the well-adjusted and the ill-adjusted lots of fishes on a given background was great enough to be visible or even conspicuous to the human eye. To what extent much less discernible differences in shade would be of survival value is a question upon which we have no evidence. But it seems quite probable that, considered statistically, even scarcely perceptible differences of this sort would be sufficient to affect mortality rates, were we concerned with sufficiently large populations. If this is true, it is probable that small differences in the rate or the extent of the capacity of these animals for color adjustment would be of selective value, and therefore of evolutionary significance. Any one who has experi-

mented in this field knows not only that different species of fishes, but different individuals of the same species, show marked differences in the perfection of this function. We thus have some of the essential conditions for the evolution of this mechanism through natural selection.

Another series of experiments (9–12) was attempted with a very different class of predators. Eight specimens of the fresh-water black-bass (*Micropterus salmoides*)[13] were used, four in each tank. These experiments were conducted under such unsatisfactory conditions that no detailed report upon them seems worth while. Owing to our allowing an insufficiently long period of "conditioning" for the *Gambusia,* and to the sickness and death of many specimens from overcrowding during transit to the experimental tanks, later identification of the "whites" and "blacks" was found to be impossible in many cases. The results, on the whole, are nearly valueless, and they certainly give no clear evidence of selection in relation to shade. Perhaps this was not to be expected in any case, for the reason that the bass almost invariably approached the "mosquito-fish" from below, the latter commonly swimming at the surface while the bass were in the tank.

It is planned to continue experiments in which fishes will be used as predators, since the chief enemies of many small species are probably the larger fishes. So far as these last may approach their prey from above, or even from the side, it is likely that homochromic (cryptic) adjustments may at times be of vital importance.[14] But experimental tests are called for.

SUMMARY

Small fishes (*Gambusia patruelis*) were kept in black and in white aquaria for some weeks, until their pigmen-

[13] These I owe to the kindness of Mr. E. H. Glidden, of the California Fish and Game Commission.

[14] Longley (1917) has furnished considerable observational evidence that this may be true.

tal differences had become sufficiently fixed to be discernible after a sojourn of a day or more upon a common background.

Equal numbers of such fishes were placed together in one or another of two large wooden tanks, one black, the other nearly white.

Here they were exposed to the attacks of fish-eating birds, including both a diving and a wading species. The number of fishes of each color (shade) which were eaten or injured were counted and tabulated (Tables 1 and 2).

The results of these tests are indicated in the italicized passages on pages 257, 260, 262 and 263.

It is concluded that the chromatic adjustments of fishes to their backgrounds may be of vital importance in protecting them from predators, despite the circumstantial evidence and theoretical objections which have been offered in disproof of this conclusion.

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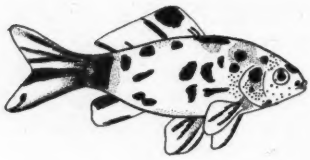
THE DEVELOPMENT OF HEREDITARY COLOR PATTERNS IN FISH[1]

PROFESSOR H. B. GOODRICH
WESLEYAN UNIVERSITY AND THE MARINE BIOLOGICAL LABORATORY

ONE of the most obvious methods of approaching the problem of the interrelation between the gene and the inherited character is to study the embryonic and later development of Mendelian characters. In evaluating such an endeavor one is at once confronted by the well-known generalization of geneticists which states that a single gene may influence many characters and that a single character may be the product of the interaction of many genes. The recognition, however, of this proposition need not be taken to vitiate the value of such attempts but rather to indicate the complexity of the problem. The varied studies relating to the development of sex characters present the most successful synthesis thus far obtained in this field. These results well illustrate the complex interplay of developmental processes that are involved. In all probability embryonic segregation, embryonic induction, cellular migration, cell differentiation and the complex interaction of hormones are all concerned in steps that bring about the end result.

In this paper it is desired to call attention to the possibilities in the study of the development of color patterns as another route of approach to the central problems of embryonic differentiation and the action of the genes. Chief attention will be given to the development of Mendelian color patterns in fish. The selection of this type of character and class of animals offers certain advantages. Pigments are easily recognized and individual chromatophores can be readily studied in the developing embryo. It is easy to observe the embryonic development of fish from start to finish.

[1] The original work reviewed in this paper that has been carried out by the author and collaborators has been made possible by grants from the Denison Foundation for Biological Research at Wesleyan University.



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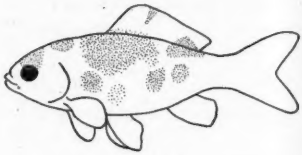

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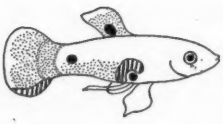

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EXPLANATION OF PLATE

FIG. 1. The common shubunkin, a variety of *Carassius auratus*, showing pattern of melanophores (solid black) and gold areas (stippled) $\times \frac{1}{2}$.

FIG. 2. A gold and white goldfish, *Carassius auratus*, stippled = gold, $\times \frac{1}{2}$.

FIG. 3. Variegated medaka, *Oryzias latipes*, showing pattern of melanophores (coarse stippling) on yellow body (fine stippling). Slightly less than life size.

FIG. 4. Rubra platy, *Platypoecilus maculatus* (after Gordon), showing macro-melanophores or spots controlled by the dominant sex-linked factor Sp. and micro-melanophores or stipples controlled by the dominant autosomal factor St. Slightly less than life size.

FIG. 5. A male guppy, *Lebistes reticulatus* (after Winge), showing the color patterns, coccineus, vitellinus and maculatus. X co, vi. Y ma. Solid black = black, stippled = yellow, crossed barred = red. Slightly more than life size.

FIG. 6. The zebra fish, *Brachydanio rerio*, showing stripes. $\times 7/10$.

The Medaka, Oryzias Latipes

A genetic analysis of color inheritance of the Japanese fish *Oryzias* (*Aplocheilus*) *latipes* has been made by Aida (1921). The genotypes of the various color phases are as follows:

BBRR —Brown (wild type)
bbRR —Red (yellow)
BBrr —Blue
bbrr —White
B′B′RR—Variegated black on yellow
B′B′rr —Variegated black on white

A study of the histology of these forms shows that the color effects are due to two types of chromatophores—the black cells or melanophores and the yellow cells or xanthophores. The color of the brown variety is due to the combined effect of both types of cells. In the yellow fish the xanthophores are normally developed, but the melanophores are apparently few in number. Studies (Goodrich, 1927) have, however, shown that undeveloped melanophores or melanophores incapable of complete development are present in normal numbers. The use of adrenalin causing a concentration of melanin within the cells reveals the presence of this full complement of otherwise unrecognizable melanophores. It is therefore clear that this recessive character differs from its dominant allelomorph in the relative absence of melanin in the chromatophores and not in the complete absence of these cells. A further analysis using the "Dopa" reaction (Goodrich, 1933) has shown that the necessary oxydase is present in these pale cells, but that the chromogen—probably tyrosine—is much reduced in quantity or absent. This leads to the conclusion that the presence of the dominant gene is necessary for the extensive accumulation of the chromogen within the cell in contrast to the double recessive condition where only a minute amount is laid down. There then appears to be a quantitative difference between the action of the dominant gene and the recessive gene. If the genetic analysis

had been restricted to uniformly colored types it might have been permissible to suggest that the difference between the two types was due to the presence and absence of some circulating hormone. The occurrence, however, of dark and light areas in the variegated types (Fig. 3) shows that the differentiating factor must be located within the cells, because a circulating hormone would affect like cells in the same fashion and therefore could not alone produce a pattern. The fact also that the "Dopa" treatment (which supplies a chromogen) brings about the development of pigment only in chromatophore-like cells indicates that the necessary oxydase is strictly limited to the appropriate cells. These observations then show that the pattern is due to differences in the chromogen producing powers of individual cells and not to hormones.

Studies (Stockard, 1915) on Fundulus have shown that chromatophores in fish arise from wandering mesenchyme cells that have their origin in the region of the closing blastipore and the mesenchyme cell mass lying within the embryo. In Fundulus unpigmented but recognizable cells migrate by amoeboid motion from these areas and later assume pigment. In Oryzias Mendelian phenotypes may be distinguished when this pigment first appears. The evidence from Fundulus indicates that the prospective fate of such cells is determined before the pigment is formed. We are therefore dealing with a case of "Chemo-differentiation" (Huxley, 1924). It then seems reasonable to advance the hypothesis that an embryonic segregation of melanophore-producing cells has occurred, and these cells migrate to various areas. In variegated fish there has evidently been a further segregation of cells which have the power to produce the full amount of melanin from those capable of forming only a small amount of that pigment. These cells then migrate and by their aggregations mark the fish with the irregular, apparently chance patterns found in these types.

The Goldfish, Carassius Auratus

A sharp contrast to the above described method of color pattern formation is found when it is contrasted with processes operating in the goldfish. Chen (1928) and Berndt (1928) have found that the ordinary goldfish (TT) crossed with an unpigmented type (T′T′) (the colorless shubunkin) yield a heterozygous form showing irregular mottling and known as the ordinary shubunkin or calico fish (Fig. 1). Berndt (1925) and Fukui (1927) have found that the ordinary goldfish attains its final coloration by a process of destruction of pigment cells. These goldfish are usually dark brown until about three months of age, when they begin to assume the red color. The brown appearance is due to the presence of both melanophores and xanthophores. The transformation is due to the disintegration of the melanophores. This destruction of cells proceeds from various centers and sweeps like a wave through the dermis of the skin. The conditions suggest those described by Il'in (1928) in the guinea pig. Red and black fish are those in which the process has not been completed. Later the red cells may be similarly destroyed, but this second phase rarely goes to completion and ordinarily the result is a pattern of red and white (Fig. 2) or of red and silver. In the case of the colorless shubunkins the depigmentation is initiated at a much earlier period—about one week after hatching. In the heterozygous form (TT′) an intermediate condition exists. There is great variability, and observations and counts of cells seem to indicate a conflict of opposing tendencies or processes. These are melanophore production and melanophore destruction (Goodrich and Hansen, 1931). The color patterns in goldfish are produced by the localized destruction of a previously established uniform color. It is also apparent that the destructive influences are operative only at certain periods of development. If scales bearing melanophores are transplanted from a black to a red area during depigmentation in the ordinary goldfish the melanophores will disintegrate in the red areas (Goodrich and Nichols,

1933). On the other hand, in the adult shubunkin in which the color pattern has been completed it is found that colored scales, red or black, may be shifted to colorless areas and retain their pigment. This is also true of the gold and white fish. It is then apparent that the destruction is limited to certain periods in the life history of the individual.

THE ZEBRA FISH, BRACHYDANIO RERIO

An intermediate mode of pattern formation probably exists in the tropical aquarium fish, *Brachydanio rerio,* otherwise known as the zebra fish due to its marked stripings (Fig. 6). In this form the regions of the future light stripes are first occupied by a sparse population of melanophores which later disintegrate (Goodrich and Nichols, 1931). The regions, however, of the black stripes have from the beginning a more dense population of melanophores. Therefore in this case the definitive pattern is formed when the pigment is first laid down, but the pattern is accentuated by the later destruction of melanophores. Conditions controlling the localization of stripes are unknown.

THE MEXICAN KILLIFISH, PLATYPOECILUS MACULATUS

The series of studies by Bellamy, Gordon and Fraser and Gordon (see Gordon, 1931a, for references) have shown the existence of a notable series of color patterns in the Mexican fish, *Platypoecilus maculatus.* In this case different effects are produced in part by different types of cells—the micro-melanophores and the macro-melanophores (Fig. 4). The stippled condition is due to the micro-melanophores and spots to the macro-melanophores. The patterns known as one spot, twin spot, crescent and moon are due to the aggregation of micro-melanophores in well-defined areas at the base of the caudal fin, whereas the Nigra or black pattern is formed of macro-melanophores. Observations by Gordon show that there are no colorless melanophores such as are found in Oryzias. It may be that in these fish there are embryonic conditions which influence the degree of

multiplication of melanophore-forming cells. In one case the presence of the gene for micro-melanophores apparently interacts with the gene for the Nigra or black type by producing an extension of the black band (Gordon, 1928). The remarkable effects produced by the interspecific crosses between Platypoecilus and Xiphophorus have been extensively studied by Gordon, Kosswig and others (See Gordon, 1931b, for references). Here the presence of genes controlling the development of macromelanophores in Platypoecilus combined with unknown factors in Xiphophorus result in melanotic overgrowths which often become destructive tumors. Here without doubt inherited factors in some way influence the extent of multiplication of the melanogenic cells.

The Guppy, Lebistes reticulatus

The studies of Schmidt and Winge on *Lebistes reticulatus* have revealed an extraordinary assemblage of color patterns, chiefly in the form of spots of varied colors and each apparently largely controlled by independent genes (Fig. 5). This then is a case where chromatophores segregate or develop in definite predetermined regions due to unknown causes. The slight evidence of sex reversion reported by Blacher (1926a, b) indicates that the uniformly colored females may assume male coloration owing to changes in the gonad. This leads to the suspicion that an invisible pattern of chromatoblasts exists in the female which may be developed by the action of internal secretions much as a photographic plate is developed by chemical treatment, or by withdrawal of an inhibiting hormone as suggested by Zahl and Davis (1933) in the case of Amia.

The Siamese Fighting Fish, Betta splendens

Preliminary studies on *Betta splendens* (Goodrich and Mercer, 1934) indicate that the green and blue forms are genetically different. Histological observations show that these colors are probably due to the refraction of light from delicate plate-like crystals and the thick-

ness of these crystals determines whether the refracted light is green or blue.

HYBRIDIZATION OF FUNDULUS AND SCOMBER

The older studies dealing with interspecific hybridizations among teleosts have yielded interesting results. The cross between the male mackerel, *Scomber scombrus,* and the female of *Fundulus heteroclitus* as reported by Newmann (1918) is peculiarly suggestive. Both fish have melanophores which, however, differ in form. Also the mackerel has striking green chromatophores and Fundulus has reddish brown chromatophores. The hybrid embryos in some cases show a complex of paternal green chromatophores and maternal red brown chromatophores and melanophores which are either intermediate in type bewteen the parental forms or approximate one or the other. Here, then, there apparently exists a peculiar situation in that the hybrid parentage sometimes shows its effect within single cells and sometimes by the production of the two parental types of cells within the single embryo.

COMPARISONS AND DISCUSSION

This survey of conditions found in fish may well serve to illustrate the complexity and variety of formative processes that may operate in color pattern formation. In Oryzias there may be embryonic segregation of cells having different capacities of melanin formation followed by cell migration. In Carassius there is the partial destruction of a uniform color; in Brachydanio both of these two first-named processes occur; in Platypoecilus there occurs the formation of two different types of melanophores and their accumulation in definite areas in certain types; in Lebistes there is a similar segregation of varied types of chromatophores in definite predetermined areas and in Betta there occurs the formation of crystals refracting different colors. In all the abovementioned cases, except in Brachydanio, there is evidence that the color patterns are Mendelian characters.

It is worth while to call attention to one or two examples from other classes of vertebrates to point out still different modes of pattern formation. The experimental work on sex plumage in fowls (see reviews by Danforth (1932) and by Domm, Juhn and Gustavson (1932) has shown that a banded pattern may be produced by an artificially controlled, rhythmic discharge of hormones into the circulation. The injection of the ovarian hormone produces a salmon-colored band on a feather otherwise black. In this manner stripes may be formed in a fashion impossible in the zebra fish. The hormones act on feather-forming cells which are engaged in secreting a non-living substance, which is then removed from influences emanating from the circulation. After this a different color may be laid down in the succeeding part. Obviously stripes of the zebra fish or of a zebra which are formed in regions continuously subject to any circulation-borne influence can not be produced by the type of mechanism operating in the fowl.

Somatic mutations may very rarely be the cause of individual variations of color patterns in mammals (Wright and Eaton, 1926). Wright's hypotheses in regard to the mechanism of color formation in the guinea pig (Wright, 1917) are suggestive of modes of interaction of genes.

A working hypothesis framed in accordance with ideas prevalent among embryologists may be suggested with special reference to the development of the color pattern in Oryzias. Here we are concerned with the three different effects produced respectively by the genes B, B′ and b as follows: Gene B induces formation of full complement (n) of melanophores; Gene B′ induces formation of approximately $\frac{n}{2}$ normal melanophores and $\frac{n}{2}$ "colorless" melanophores; Gene b induces the formation of n "colorless" melanophores. In view of the generally accepted hypothesis that all cells in the same animal have normally the same genic complex we can not assume that the gene

B′ acts by a single step to produce both the normal and the colorless chromatophores which are both found in the same phenotype. Similar nuclei acting to produce diverse results must be located in different cytoplasmic environments. The formations of such different cytoplasms must be referred to an earlier period of embryonic segregation. At such a time we may suggest that gene B may form enzymes which reacting with the cytoplasm form a differentiated cytoplasm (chemo-differentiation) which is later cut off by cell division. Gene B′ will produce less of such material and gene b least. Fewer cells containing this differentiated cytoplasm will then be found in phenotype B′ and almost none in b. Nuclei lying within these differentiated cells or possibly hormones from a more remote source will induce the formation of the chromogen which, acted upon by the oxydase present in all chromatoblasts, will produce the melanin. In type B all melanoblasts can produce the full amount of melanin and cover the whole body; in B′ only about half of the cells can produce a recognizable amount of melanin and these migrate irregularly over the body; and in b practically no cells can form any but the minimum amount of melanin. A modified form of this hypothesis might be applied to the formation of the micro- and the macro-melanophores in Platypoecilus. As indicated above, however, it can not be applied to explain the color patterns in the goldfish. Fukui (1930) suggests that lysins present in lymph spaces may cause the disintegration of chromatophores but apparently such an agent is present for only a limited period in the life cycle. No satisfactory hypothesis has been found to explain the delimitation of precise figures or stripings such as are found in Lebistes or in Brachydanio.

It is a striking fact that there have been found notable divergencies in the mode of color pattern formation in every species of fish examined, and therefore it is impossible to generalize in regard to the mechanism of pattern formation.

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SHORTER ARTICLES AND DISCUSSION

QUANTITATIVE CHARACTERS IN RECIPROCAL HYBRIDS

In general, as a result of the preponderant influence of genes on the development of organisms, reciprocal hybrids, both inter- and intra-specific, are alike. There are, nevertheless, some exceptions. For example, a disproportionate maternal influence was patent in the vestigial-normal winged heterozygotes in Drosophila reported by Hersh and Ward (1932). East (1934), in a comprehensive survey of the literature, has brought together a number of examples among plants. Verified differences in reciprocal mammal crosses are very rare, the most clearly demonstrated instances being the marked contrast in the incidence of spontaneous tumors in the offspring from the two types of matings between "high" and "low" tumor strains of mice, reported by Little (1933), and the similar results in the inheritance of spontaneous mouse leukemia, announced by MacDowell (1935).

An obvious explanation for excessive maternal influence in hybrids is the postulation of cytoplasmic inheritance, although the existence of such a hereditary mechanism is as yet unproved. Castle (1933) has suggested that cytoplasmic differences may be partly responsible for quantitative racial or specific characters. If size inheritance is plasmatic in any degree there should be a certain amount of dissimilarity in reciprocal hybrids between forms distinguished by quantitative differences. It might be anticipated, then, that long-established specific characters which exhibit blending inheritance usually explained as the result of the action of many genes, would show a maternal influence more strongly than recent superficial characters conditioned by single mutant genes.

During the past few years a considerable amount of data has been amassed in this laboratory on crosses between two species of mice: *Mus bactrianus* from China and the ordinary house mouse, *Mus musculus*. Included are some data on reciprocal hybrids.

These two murids, although given specific rank by taxonomists, mate readily, producing hybrids of which both sexes are fertile. Because of the smaller and less frequent litters, later maturity and relative scarcity of *bactrianus* as compared with *musculus*

females, hybrids from Chinese dams were produced in much smaller numbers than the reciprocals.

Since descriptions of most have been published elsewhere (Green, 1931a, b) diagnostic features of the two forms of mice may be summarized as follows: *Mus bactrianus* is much smaller, has a relatively shorter tail, fewer caudal vertebrae, a baculum (os penis) of slightly different size and proportions and smaller litters than its larger congener. It is with these quantitative characters—some of which are characteristic for the species—in the reciprocal hybrids that this note is concerned.

Since the mice were produced during the course of two or three investigations—in not all of which the same diet was used—and the numbers in each were sometimes unfortunately small, several mathematically crude steps were necessary to combine the diverse series of weights to obtain the advantage of larger numbers. The mean of all females combined was computed and divided by the comparable mean in each of the three investigations; the individual weights in each were then multiplied by the appropriate quotient. The same method was utilized for the two experiments involving males. No such procedure was considered necessary in the case of the other morphological characters, since their manifestation in adults is probably almost entirely independent of weight. Green and Fekete (1933) have demonstrated such independence in both relative tail length and number of caudal vertebrae. In the data summarized in Table 1 only nulliparous females are included.

TABLE 1

Mating	Sex	No.	Mean weight (181st day)	Difference / Standard error
musculus ♀ × *bactrianus* ♂	♂	75	24.54 ± .36 grams	−0.9
bactrianus ♀ × *musculus* ♂	♂	26	25.10 ± .52 "	
musculus ♀ × *bactrianus* ♂	♀	64	20.76 ± .25 "	4.5
bactrianus ♀ × *musculus* ♂	♀	38	18.50 ± .42 "	

These figures clearly show the difference in the males to be insignificant; in fact, the trend is toward the paternal rather than the maternal parent, so plasmatic inheritance obviously can play no part. In the females the data appear quite otherwise, with the offspring from the large female parent much heavier than the reciprocals. Whether this difference is as highly sig-

nificant as it seems remains questionable; when the abnormally small Ann Arbor $\frac{bactrianus}{musculus}$ females (Green, 1931a) are excluded the discrepancy is not nearly so great.

The mean ratio of tail length to body length was the criterion used for relative tail length. In computing this value and its standard error the following formulae, for which we are indebted to Professor Sewall Wright, were employed:

$$\overline{R} = \frac{m_1}{m_2}\left[1 + \left(\frac{\sigma_2}{m_2}\right)^2 - \left(\frac{\sigma_1}{m_1}\right)\left(\frac{\sigma_2}{m_2}\right) r_{12}\right]$$

$$E_{\overline{R}} = \frac{m_1}{m_2}\sqrt{\left(\frac{E_{m_1}}{m_1}\right)^2 + \left(\frac{E_{m_2}}{m_2}\right)^2 - 2\left(\frac{E_{m_1}}{m_1}\right)\left(\frac{E_{m_2}}{m_2}\right) r_{12}}$$

M_1 and m_2 represent mean tail length and mean body length, respectively.

The computed values for reciprocal hybrids are listed in Table 2.

TABLE 2

Mating	No.	$\overline{R}$	Difference / Standard error
musculus ♀ × *bactrianus* ♂	104	.940 ± .004	1.5
bactrianus ♀ × *musculus* ♂	48	.929 ± .006	

Although hybrids from short-tailed dams have slightly shorter tails, relatively, the difference is too slight to be of significance.

The number of caudal vertebrae is probably a diagnostic character in the two species, for the number in each is remarkably constant with no overlapping whatever. Since it is sometimes difficult to distinguish the fourth sacral vertebra from the first caudal, the latter, in counting, was taken as the fifth vertebra, beginning with the first sacral which can usually be easily determined. All specimens included in the computations were cleared and stained with alizarin red S, following Dawson's (1927) method, so the determination of the numbers is probably as accurate as possible and considerably more reliable than figures given in an earlier paper.

Again the difference in reciprocal hybrids is of no importance and, in this case, even in the direction opposite to that expected if plasmatic inheritance were indicated.

Still another trait in which *Mus musculus* and *Mus bactrianus* differ is size of litters, those of the latter averaging smaller. The data presented in Table 4 amply demonstrate that no difference exists in litter size of reciprocal F_1 females.

TABLE 3

No. caudal vertebrae	*bactrianus*	*musculus*	$\frac{musculus}{bactrianus}$	$\frac{bactrianus}{musculus}$
24	1			
25	30			
26	15		1	
27			7	2
28			47	14
29		1	24	9
30		32		
31		23		
No. mice	46	56	79	25
Mean vertebral number	25.3 ± .1	30.4 ± .1	28.2 ± .1	28.3 ± .1

TABLE 4

Female parent	No. litters	Mean litter size	$\frac{Difference}{Standard\ error}$
$\frac{musculus}{bactrianus}$	82	6.82 ± .21	−.07
$\frac{bactrianus}{musculus}$	76	7.04 ± .24	

TABLE 5

Mating	No.	Av. length	Av. width	Av. ratio $\left(\frac{width}{length}\right)$
bactrianus ♀ × *bactrianus* ♂	22	25.7	8.0	.31
musculus ♀ × *musculus* ♂	24	25.2	8.1	.32
musculus ♀ × *bactrianus* ♂	38	26.8	9.1	.34
bactrianus ♀ × *musculus* ♂	12	28.0	9.9	.35

Size and shape of the baculum (os penis) has been used as a taxonomic criterion in certain groups of small mammals, for example, the chipmunks (Howell, 1929), so representatives of the two species of mice were cleared and stained with alizarin red S preparatory to measurement with an eyepiece micrometer.

The means, in micrometric units, show no evidence of maternal inheritance, although the numbers are too small to be relied on (Table 5).

It is of interest in connection with the failure to detect any consistent differences in morphological characters in reciprocal hybrids between *Mus bactrianus* and *Mus musculus* that females of the two types of F_1 offspring exhibited such marked disparity in incidence of spontaneous mammary tumors (Little, 1933).

C. V. GREEN

ROSCOE B. JACKSON MEMORIAL LABORATORY
BAR HARBOR, MAINE

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GAMETIC ELIMINATION IN CROSSES BETWEEN SELF-STERILE SPECIES

SINCE the demonstration by East and Mangelsdorf (1925) of self-sterility brought about through gametic elimination, similar systems have been reported in numerous genera of the flowering plants. While genetically it is not the only device by which self- and cross-sterility is regulated, it is apparently among the commonest. In crosses between such self-sterile species the gametic elimination might theoretically have perceptible morphological consequences through linkage between the self-sterility allelomorphs and genes affecting size, shape, color, etc. One of the most interesting cases would be provided by a cross between

two diploid self-sterile species. The possible genetic set-up in such an experiment is as follows:

In a cross between two self-sterile diploid species we may diagram the self-sterility allelomorphs of the first species as S_1S_2 and those of the second as s_1s_2. It will further simplify the discussion if we refer to the first species as "major" and to the second as "minor." In the F_1 there should be four intra-sterile, inter-fertile classes of equal size, S_1s_1, S_1s_2, S_2s_1, S_2s_2. There are six possible combinations between these four classes, as follows:

$S_1s_1 \times S_1s_2$ ..... gametic elimination of S_1 from major
$S_1s_1 \times S_2s_1$ ..... gametic elimination of s_1 from minor
$S_1s_1 \times S_2s_2$ ..... complete recombination
$S_1s_2 \times S_2s_2$ ..... gametic elimination of s_2 from minor
$S_2s_1 \times S_2s_2$ ..... gametic elimination of S_2 from major
$S_1s_2 \times S_2s_1$ ..... complete recombination

As regards the self-sterility locus, there are therefore three types of F_2's, those in which there is complete recombination, those in which there is elimination of a gene from major, and those in which there is elimination of a gene from minor. The elimination would apply not only to the self-sterility locus but to linked neighboring loci, some of which would certainly affect visible characters, such as size, shape and color. If the two species were at all distinct morphologically one should be able to detect at least a difference between the averages of the three F_2's; one intermediate, one closer to major and one closer to minor. It is to be hoped that some one may have the material which will make it possible to test the matter experimentally.

EDGAR ANDERSON

ARNOLD ARBORETUM
HARVARD UNIVERSITY

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LINKAGE RELATIONS IN THE A–Rg GROUP IN MAIZE[1]

THE finding of the dominant ragged (Rg_1) character in maize a few years ago has facilitated appreciably the mapping of the genes in group 3. Brink and Senn[2] found that Rg_1 was linked

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 185. Published with the approval of the director of the station.

[2] *Jour. Heredity*, 22: 155–161.

not only with anthocyanin (A_1) and tassel seed (ts_4) but also with dwarf (d_1). Up to that time it had been assumed that d_1, together with crinkly (cr_1), represented a distinct linkage group. It is a fortunate circumstance that Rg_1 occupies a locus in the central section of the group, as the latter is now known. Since the mutant character is dominant, coupling series may be obtained in F_1 with unplaced recessive genes, and a backcross test for linkage made directly over what is probably the maximum range when only one marker is used. Using Rg_1 in this way, several new characters have been tested for linkage in group 3, and two located therein. These characters are liguleless-2 (lg_2) (Brink, 1933) and pale midrib (*pm*) (unpublished). It is probable also that ramosa-2 (ra_2) is in chromosome 3, although the present evidence is not decisive.

Concurrent with the attempts to add further to the number of usable genes in group 3, efforts have been made to determine the relative positions of the characters which have been placed there. The present paper embodies a summary of this latter work.

In the tabulations below, the left-hand column shows in each case the respective regions in which crossing over occurred, 0 being employed to designate the grandparental combinations. In the next two columns the frequencies of the several classes are shown in the usual systematic order, the first value relating to plants whose genetic formula begins with the dominant member of the first allelomorphic pair. Thus, according to the first table, there occurred 42 *A*$_1$ *na Ts*$_4$ *Rg*$_1$ and 81 *a*$_1$ *Na ts*$_4$ *rg*$_1$ individuals, totaling 123 crossovers in region 1 of this cross. Below each table the order of the genes tested is given, together with the map distances between them as indicated by these experiments.

I. (*A*$_1$ *Na ts*$_4$ *rg* . *a*$_1$ *na Ts*$_4$ *Rg*$_1$) × *a*$_1$ *na ts*$_4$ *rg*$_1$

0 :	235 +	216 =	451
1 :	42 +	81 =	123
2 :	140 +	105 =	245
3 :	24 +	27 =	51
1, 2 :	32 +	56 =	88
1, 3 :	4 +	9 =	13
2, 3 :	14 +	3 =	17
1, 2, 3 :	2 +	3 =	5
		Total	993

a$_1$ 23 *na* 36 *ts*$_4$ 9 *Rg*$_1$

II. $(A_1\ lg_2\ rg_1\ .\ a_1\ Lg_2\ Rg_1) \times a_1\ lg_2\ rg_1$

0 : 400 + 303 = 703
1 : 196 + 210 = 406
2 : 76 + 62 = 138
1, 2 : 39 + 29 = 68

Total 1315

a_1 36 lg_2 16 Rg_1

III. $(A_1\ Ba_1\ Rg_1\ .\ a\ ba_1\ rg_1) \times a_1\ ba_1\ rg_1$
(ba_1 = barren stalk–1)

0 : 10 + 10 = 20
1 : 9 + 9 = 18
2 : 6 + 4 = 10
1, 2 : 0 + 1 = 1

Total 49

a_1 39 ba_1 22 Rg

IV. $(Lg_2\ d_1\ .\ lg_2\ D_1) \times lg_2\ d_1$

0 : 81 + 81 = 162
1 : 59 + 37 = 96

Total 258

lg_2 37 d_1

V. $(Rg_1\ d_1\ .\ rg_1\ D_1) \times rg_1\ d_1$

0 : 135 + 156 = 291
1 : 36 + 58 = 94

Total 385

Rg 24 d_1

Summary

The linkage relations indicated below are established for group 3 in maize. The order of the genes involved in each experiment is shown and the amounts of crossing over between successive marked loci.

1. a_1 23 *na* 36 ts_4 9 Rg_1.
2. a_1 36 lg_2 16 Rg_1.
3. a_1 39 ba_1 22 Rg_1.
4. lg_2 37 d_1.
5. Rg_1 24 d_1.

R. A. Brink

Department of Genetics
University of Wisconsin

A CASE OF FUNCTIONAL HERMAPHRODITISM IN THE SEA-URCHIN, *ARBACIA PUNCTULATA*, AND AN ESTIMATE OF THE SEX-RATIO

RELATIVELY few examples have been reported of the curious phenomenon of hermaphroditism in sea-urchins. Gray described in the Neapolitan *Arbacia pustulosa* an individual exhibiting four female gonads, and the fifth partly female, but preponderantly male in appearance. The spermatozoa appeared normal, but were motionless, or swam feebly. Sectioning revealed no ovarian tissue in the entire gland. A case of hermaphroditism in *Strongylocentrotus lividus* was also described. This individual contained three completely female gonads, and two which liberated both eggs and sperm, which were fertile *inter se*. Gadd has instanced in *Strongylocentrotus droebachiensis* an animal with one male gonad and four female gonads; he also cites several references to the recorded cases of hermaphroditism in echinoderms. One of the most interesting is that of the regularly occurring protandrous hermaphroditism of *Asterina gibbosa* found at Roscoff, a condition quite unique amongst asterids, and described by Cuénot.

During the latter part of the season of 1934, while working at Woods Hole with *Arbacia punctulata*, an urchin three centimeters in diameter at the equator was opened. This disclosed a genital apparatus consisting of four testes and one ovary which, as regarded respective coloration, appeared perfectly normal. The ovary shed many eggs (estimated at about 1,000) and when inseminated with sperm from the same animal, displayed nearly 100 per cent. fertilization, with well-defined membranes. The cleavages were abnormal, both in regard to tempo and morphology. In general, the first cleavage was considerably delayed, and in numerous embryos the early cleavage planes were imperfect or incomplete in extent. At 9:38 A. M. (September 20)—about 78 minutes after fertilization—some eggs were in the two-cell stage, some in four cells, others with abnormal cleavage, while most of the eggs remained unsegmented at this time. About two hours later, all the cells had undergone cleavage. (Eggs from normal urchins, which had been brought into the laboratory and kept in tanks for several weeks and thus inhibited from shedding genital products, developed normally). At 23° C. the typical periods required for first and second cleavage are

50 and 78 minutes, with the third cleavage at 103 minutes (E. N. Harvey). Of the total number of embryos, a large proportion gave rise, however, to viable free-swimming blastulae, and about 30 per cent. gastrulae. It was not possible to compare development of selfed and crossed eggs, for on opening the animal, one testis burst and shed sperm around the ovary.

Slightly earlier in the season, a similar urchin had been opened, in which the ovary-like organ contained no eggs, but large quantities of pigmented amoebocytes. In late August, or early September, when many of the animals have already shed their genital products, one occasionally finds a specimen of either sex in which four of the gonads are diminutive in consequence of having shed their cells, whilst the remaining one is swollen with ripe genital products and liberates them readily when the surface is pierced with the point of the scissors. Females which have shed their eggs frequently have ovaries which are distinctly lighter than the normal purplish-red, owing, apparently, to the loss of echinochrome, which is abundant in the eggs themselves; in fact, they then appear much like testes. Depleted testes, however, usually contain a modicum of sperm which may be demonstrated by pressing the testis, whereupon a localized creamy ooze of spermatozoa issues forth.

It is not known whether this sea-urchin is an example of true sex reversal, or a condition existing *ab initio* in this particular animal, and arising in the course of its embryogeny. We have the now classical example of a case of sex reversal in poultry, reported by Crew, in which the reversed female sired both male and female offspring. Sex reversal in fishes and amphibia also have been described by Crew in his book, where references to the original literature may be found. Baltzer's observations on *Strongylocentrotus lividus* and *Echinus microtuberculatus* led him to conclude that in echinoids the female is the heterogametic sex, and the male homogametic, but Tennent pointed out that in *Hipponoë esculenta,* half of the sperm bear a heterochromosome. Thus, as Pinney stated, "in the light of our present knowledge of the occurrence of the accessory chromosome in other groups, these facts present an interesting anomaly." Tennent estimated that *Arbacia punctulata* had about forty chromosomes.

The writer is not aware of any report on the sex ratio in this species; a simple method of distinguishing the sexes superficially is not known. No grossly apparent secondary sex difference was

observed on casual examination of the genital pores and plates, nor of the corrugations of the spines. During July, August and early September of 1934, while the eggs were being used for experimental purposes, a record was kept of the sexes of all animals opened. The urchins had been collected from various points in the Woods Hole region, and ranged in size (equatorial diameter, exclusive of spines) very roughly from two to three and one half centimeters. Of 2,358 such urchins inspected, 1,161 were males, and 1,197 females, giving a sex-ratio of 1:1.03. When grouped according to size, approximately the same ratio prevails. It remains to be demonstrated that this ratio is representative of the ocean population of *Arbacia punctulata* in general.

HERBERT SHAPIRO
National Research Fellow in the Biological Sciences

MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MASS., AND
BIOLOGICAL LABORATORY
PRINCETON UNIVERSITY

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